

**HYPERINSULINAEMIA, INSULIN RESISTANCE AND
ENDOGENOUS FIBRINOLYSIS IN ISCHAEMIC HEART DISEASE**

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ABSTRACT

The work presented in this thesis has explored the hypotheses that disturbances of insulin and of endogenous fibrinolysis are a feature of patients with established ischaemic heart disease and investigated the potential manipulation of these by an inhibitor of the angiotensin converting enzyme.

Hyperinsulinaemia implies either abnormally elevated fasting concentrations of insulin, or an increased insulin response to a stimulus - usually an orally or intravenously administered glucose load. Insulin resistance describes a state in which the biological effect of insulin on glucose metabolism is less than it should be, and this usually leads to a chronic compensatory hyperinsulinaemia.

The possibility that the presence of either a previous myocardial infarction or of heart failure might influence the development of hyperinsulinaemia was studied. Only patients with heart failure showed fasting hyperinsulinaemia, whereas the increased insulin response to an oral glucose load in those with heart failure or previous myocardial infarction was similar, and greater than the response in patients with stable angina. Hyperinsulinaemia has been previously associated with impaired peripheral muscle glucose uptake and metabolism and might contribute to the development of muscular fatigue on exertion in patients with previous myocardial infarction or with heart failure.

Amongst patients with ischaemic heart disease who had a normal fasting plasma glucose, one fifth had impaired glucose tolerance on formal testing and this group exhibited significantly greater fasting and stimulated hyperinsulinaemia.

Including all patients, there was an inverse relationship between left ventricular ejection fraction and fasting plasma insulin concentration.

The angiotensin converting enzyme inhibitor captopril has been shown to improve prognosis after acute myocardial infarction. The possibility that captopril might favourably influence alterations of insulin secretion or insulin resistance in patients with recent uncomplicated myocardial infarction was investigated but no significant effect could be demonstrated.

Impaired endogenous fibrinolysis has been associated with an adverse prognosis in patients with ischaemic heart disease. The effect of captopril upon tissue-type plasminogen activator and on plasminogen activator inhibitor type 1 was investigated in patients with recent uncomplicated myocardial infarction. Captopril caused a significant reduction in antigen levels of both tissue-type plasminogen activator and of plasminogen activator inhibitor type 1. This may help to explain the reduction in acute coronary syndromes that has been associated with the use of captopril following acute myocardial infarction.

The truth is rarely pure, and never simple

Oscar Wilde

The Importance of Being Earnest

Imagine

John Lennon

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DECLARATION

This thesis describes research undertaken in the Department of Cardiology at the Royal Infirmary of Edinburgh from my position as Lecturer in the Cardiovascular Research Unit of Edinburgh University during the period from 1989 to 1993. I have been fortunate in having the advice and help of several colleagues who are formally acknowledged. The substantial part of the work in this thesis has been my own and the writing of this text has been entirely my own undertaking. Some of the work presented has been published in academic journals and copies of these publications and other relevant publications of my own constitute the Appendix.

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The work represented in this thesis was carried out whilst I was Lecturer to the Cardiovascular Research Unit and Honorary Registrar in the Department of Cardiology at the Royal Infirmary of Edinburgh. It has been a privilege and a pleasure to work with colleagues in both of these departments and I wish to express my gratitude to all of them.

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CHAPTER 1

A REVIEW OF INSULIN, ENDOGENOUS FIBRINOLYSIS AND THEIR POSSIBLE IMPLICATIONS FOR PATIENTS WITH ISCHAEMIC HEART DISEASE

1.1 THE BIOLOGY OF INSULIN

1.1.1 INSULIN AND ITS SECRETION

The human insulin gene is 1500 base pairs long and is located on the short arm of chromosome 11. Insulin is a polypeptide hormone secreted by the β cells within the islets of Langerhans in the endocrine pancreas. It is synthesised as proinsulin and the pre-sequence is removed within the endoplasmic reticulum to form proinsulin (Espinal 1989).

The proinsulin molecule is transported to the Golgi apparatus, where proteolysis and packaging into secretory granules begins. Proinsulin consists of 3 units - the A (acidic) chain, the B (basic) chain, and the C (connecting) peptide. The proinsulin molecule undergoes a series of site-specific peptide cleavages that lead, via the intermediate species of 32,33 split proinsulin and 65,66 split proinsulin, to the formation of mature insulin and C peptide in equimolar concentrations.

The insulin molecule consists of the A chain, which has 21 amino acids, and the B chain, which has 30 amino acids. The molecule contains 3 disulphide bridges: 2 linking the A and B chains and an internal one in the A chain. The evolutionary conservation of this structure is remarkable and hints at the biological importance of the molecule.

The conversion of proinsulin to insulin takes place as the secretory granules mature, and usually this processing is about 95% complete prior to secretion. At the time of secretion, mature granules fuse with the plasma membrane and discharge their contents into the extracellular fluid. Insulin and C peptide are released into the

pancreatic vein, which empties into the portal vein. This is an important arrangement, since the liver is a primary site of action for insulin. Insulin has no plasma carrier protein and its plasma half-life is less than 5 minutes under normal conditions. The major organs involved in insulin metabolism are the liver and kidneys (and the placenta during pregnancy) and at least 2 enzyme systems are responsible for its metabolism. The first involves an insulin-specific protease, which is present in many tissues, and the second involves hepatic glutathione insulin transhydrogenase. Thus, the highest concentrations of insulin occur in the hepatic portal vein prior to first-pass metabolism.

Under normal conditions, a small amount of proinsulin and its split products are released with the insulin and C peptide from the secretory granules. These have a significantly longer half-life than insulin, although they have less than 5% of the bioactivity of insulin because the active site of insulin is occluded in the precursor molecule. Proinsulin and its split products may cross-react with insulin antisera, and if present in increased concentrations they may cause a radioimmunoassay for "insulin" to overestimate the bioactivity of "insulin" in plasma. The C peptide has no known biological activity and is a distinct molecule from an antigenic standpoint.

The normal human pancreas secretes 40-50 units of insulin daily, which represents about 15-20% of its stores. The most physiologically important stimulus to the release of insulin is an increase in plasma glucose. Insulin secretion is also increased by β adrenergic agonists and by chronic exposure to cortisol, growth hormone, oestrogen, progesterone and human placental lactogen. Alpha adrenergic agonists inhibit release of insulin.

There are two hypotheses that explain the mechanism by which the β cell recognises glucose, and both are referred to as the “glucoreceptor model.” The first model describes a classical membrane receptor for glucose that transmits the signal to intracellular pathways leading to insulin secretion. Unfortunately, no such receptor has been identified. Most evidence supports the alternative hypothesis, that the “glucoreceptor” is the metabolism of glucose itself. Randle recently reviewed the hypothesis and suggested that the “glucoreceptor” is the phosphorylation of glucose, which in the β cells is catalysed by glucokinase (Randle 1993). The transport of glucose into β cells and hepatocytes is by GLUT-2, an insulin independent glucose transporter protein with a high capacity, facilitating a rapid equilibration between intracellular and extracellular glucose. The first step in the metabolism of glucose in animal tissues is phosphorylation to glucose 6-phosphate, which in β cells and hepatocytes is catalysed by glucokinase, and in all other tissues by hexokinase. This is significant because the concentration of glucose at which the rate of phosphorylation catalysed by glucokinase is half the maximal rate (K_m , or the Michaelis constant) is high (6.1 - 8.9 mmol/L), which means that the rate of glucose phosphorylation varies with changes in the glucose concentration throughout the physiological range. Thus it acts as an accurate sensor by which to regulate the secretion of insulin, developing a greater stimulus with increasing levels of plasma glucose, and providing very little stimulus at basal glucose concentrations (3.8 - 5.2 mmol/L).

1.1.2 THE INSULIN RECEPTOR

The actions of insulin are mediated by an integral plasma membrane protein, the insulin receptor. The insulin receptor gene is located on the short arm of chromosome 19 and is even more highly conserved in evolution than the gene for insulin itself. The processed receptor consists of 2 α subunits, which are located on the outer surface of the membrane and contain the insulin binding site, and 2 β subunits, which traverse the membrane and are protein tyrosine kinases in their cytosolic domain. Tyrosine phosphorylation is unusual in mammalian cells (phosphotyrosine accounts for < 0.1% of the phosphoaminoacid content of normal cells), and yet it is interesting to note that, amongst others, platelet derived growth factor, angiotensin II, insulin-like growth factor I and many viral oncogene products also act on receptors which have tyrosine kinase activity. The low intracellular concentration of proteins containing phosphotyrosine suggests that they may well have a regulatory function.

Insulin receptors are found on most mammalian cells, in concentrations of up to 20,000 per cell, and often on cells not typically thought of as being insulin targets. For example, most of the insulin binding capacity of mixed leukocyte preparations resides with monocytes. The receptor is constantly being synthesised and degraded, and its half-life is 7-12 hours. The receptor is translated as a single chain peptide in the rough endoplasmic reticulum and is processed within the Golgi apparatus to form the mature α and β subunits.

When insulin binds to the receptor, several events occur: (1) There is a conformational change of the receptor; (2) the receptors crosslink and form

microaggregates; (3) the receptor is internalised; and (4) one or more signals are generated. Within the cell, insulin is degraded in the lysosome, but the receptor may be recycled to the cell surface. The number of insulin receptors on the cell surface is tightly controlled, but may be up or down regulated. Factors known to influence receptor concentration include insulin itself, physical exercise, and hormones such as corticosteroids and growth hormone.

The exact nature of the intracellular mechanisms initiated by the binding of insulin to its receptor remain obscure. Activation of tyrosine kinase leads to activation of a receptor-associated serine kinase, and it is likely that the latter initiates phosphorylation/dephosphorylation reactions leading to the generation of second messengers. The nature of the second messenger(s) is poorly understood, but one candidate is phospholipase C. Most other hormones exert their action through one of two other second messenger systems, changes in the concentrations of cAMP or of inositol triphosphate. While some of insulin's actions are associated with an increase of cAMP, this does not account for the majority.

1.2 PHYSIOLOGICAL ACTIONS OF INSULIN

Although in clinical medicine the primacy of insulin is considered to be the control of the blood glucose concentration, its actions are remarkably protean.

These include: (1) Metabolic effects - including carbohydrate, lipid and protein metabolism; (2) effects on the autonomic nervous system; (3) haemodynamic effects;

- (4) effects on electrolyte balance; (5) effects on cell replication and cell death; and (6) effects on gene transcription.

1.2.1 METABOLIC EFFECTS OF INSULIN

These may be usefully considered by the division into the postabsorptive state and the postprandial state. The plasma insulin level depends upon the blood glucose level, which in its turn depends upon three variables: (i) gastrointestinal absorption; (ii) peripheral glucose uptake and utilisation; and (iii) hepatic glucose production by glycogenolysis and gluconeogenesis. Excretion of glucose only occurs when the renal threshold is exceeded, i.e. in diabetes mellitus and sometimes during pregnancy. In the postabsorptive state, peripheral glucose uptake and hepatic glucose production are equal and this balance is principally maintained by the combination of insulin and glucagon. The plasma concentrations of glucose (low) and of non-esterified fatty acids (high) are stable.

Postabsorptive state

The term postabsorptive state refers to the period 6-12 hours after a meal during which the transition from the postprandial to the fasting state occurs. At this time, glucose metabolism continues to be the main source of energy for tissues that are obligate glucose metabolisers, principally the brain and erythrocytes, whose uptake of glucose is independent of insulin. Plasma insulin levels are low, and the insulin sensitive tissues (peripheral muscle, myocardium, liver and kidneys) generate ATP from the β oxidation of non-esterified fatty acids. In these tissues, the relationship between the metabolism of glucose and fatty acids is reciprocal; the

glucose fatty acid cycle or Randle hypothesis operates (Randle et al 1963). Put simply, this states that the oxidation of non-esterified fatty acid inhibits the uptake of glucose by insulin sensitive tissue; thereby sparing glucose for metabolism by non-insulin sensitive tissues.

Postprandial state

The postprandial response is obviously dependent upon the nature of the ingested meal. In most studies, and those described in this thesis, the traditional stimulus has been an aqueous solution of glucose. Following gastrointestinal absorption, plasma glucose rises within 10 minutes and stimulates the islet β cells to produce insulin, and suppresses release of glucagon from the α cells. Initially, when portal venous concentrations are high, some ingested glucose is used to replenish hepatic glycogen stores (insulin independent uptake), but most of the absorbed glucose load is disposed of into peripheral muscle (insulin dependent uptake). Within the muscle, glucose may undergo oxidative metabolism or non-oxidative metabolism. Oxidative metabolism initially requires glycolysis, which produces 2 pyruvate molecules from one of glucose, after which the pyruvate is converted to acetyl CoA, which undergoes complete oxidation in the tricarboxylic acid cycle. Non-oxidative metabolism occurs either when only glycolysis takes place, or if glucose is diverted into the muscle glycogen store (catalysed by glycogen synthase). Insulin stimulates these processes at three main sites: (i) the transport of glucose across the cell membrane; (ii) the activity of glycogen synthase; and, in adipose tissue, (iii) the activity of pyruvate dehydrogenase, which catalyses the conversion of pyruvate to acetyl CoA:

(i) The effect of insulin on glucose transport involves a translocation of glucose transporters from intracellular membrane to the plasma membrane, and then activation of the transporter. These are 5 isoforms of transporter (GLUT 1-5) with tissue specific expression: GLUT 1 (brain, erythrocyte); GLUT 2 (liver, pancreatic β cell); GLUT 3 (gut, kidney); GLUT 4 (skeletal muscle, adipose tissue); and GLUT 5 (gut, kidney).

(ii) Glycogen synthase activity is regulated by phosphorylation and dephosphorylation. Insulin activates the enzyme by activating glycogen synthase phosphatase and inhibiting one of the glycogen synthase kinases, cAMP-dependent kinase.

(iii) The pyruvate dehydrogenase complex in the mitochondria of adipocytes is activated by insulin. Thus, it encourages the carbon atoms of glucose to venture beyond “the point of no return” to become acetyl CoA (an irreversible reaction), from where they must either enter the tricarboxylic acid cycle or, in adipose tissue in particular, be used to synthesise fatty acids. Insulin also increases the rate of triglyceride synthesis and inhibits lipolysis. Thus, by lowering the concentration of circulating non-esterified fatty acids, the utilisation of glucose by peripheral muscle is increased, as predicted by the glucose fatty acid cycle hypothesis.

Lastly, the effects of insulin upon protein metabolism have little effect on glucose or fatty acid metabolism. In the short term (minutes) insulin stimulates amino acid uptake, and in the longer term (hours) it alters protein synthesis via direct effects on the generation and translation of mRNA.

1.2.2 NEURAL EFFECTS OF INSULIN

The modulation of insulin release by both α and β adrenergic stimulation has been described. The possibility that insulin might alter sympathetic nervous system activity has aroused interest, mainly as a possible link between raised insulin levels and increased blood pressure. Augmented sympathetic nervous system activity may also be important in ischaemic heart disease as recently reviewed by Kubler (Kubler 1992) and as witnessed in clinical practice by the widespread use of β adrenoceptor antagonists.

Pereda was the first to report that intravenous administration of insulin to the dog caused an increase in blood pressure prior to the onset of hypoglycaemia, and that, if hypoglycaemia was prevented, the effect persisted (Pereda et al 1962). In addition, it was found that smaller doses of insulin, which had no effect when administered intravenously, elicited a pressor response when infused into the carotid artery, suggesting that insulin may have a direct action on the brain. The response was antagonised by phentolamine (an α adrenoceptor antagonist) and by trimethidinium (a ganglion blocker). Further animal studies have revealed insulin-specific binding sites in the hypothalamus (Van Houten et al 1983), and also shown that intraventricular injections of insulin increase central nervous system catecholamine turnover (Sauter et al 1983).

In a study very similar in concept to that of Pereda, Rowe reported the effects of administering an intravenous infusion of insulin to 12 normal men (Rowe et al 1981). Blood glucose levels were maintained at fasting concentrations using the hyperinsulinaemic euglycaemic clamp technique (De Fronzo et al 1979; see

Chapter 2). A dose of insulin that maintained the plasma concentration in the high physiological range (~ 150 mU/L) was associated with a 50% increase in plasma noradrenaline, an increase in heart rate, but no change in blood pressure. A higher dose of insulin that raised plasma concentrations of insulin to supraphysiological levels (~ 600 mU/L), was associated with a 117% rise in noradrenaline and a significant increase in mean blood pressure. The metabolic clearance of noradrenaline was not altered by the insulin infusion.

Oral administration of 100 g glucose to 19 healthy subjects (which raised plasma insulin levels to ~ 50 mU/L) was associated with an increase of muscle nerve sympathetic activity, measured by a microelectrode positioned in the peroneal nerve (Berne et al 1989). Changes in muscle nerve sympathetic activity were apparent within 15 minutes and there were concomitant increases in plasma noradrenaline, but no change in plasma adrenaline. There was a small, but significant, increase in heart rate, but no change in blood pressure. The sympathetic nerve activity to the skin was examined in 3 subjects and showed no change, suggesting a selective augmentation of sympathetic activity. There was no evidence of increased sympathetic outflow when intravenous glucose was given in place of the oral load, and although the peak plasma insulin level was similar, the duration of increase in circulating insulin was very much less after intravenous glucose. This suggests that it was the hyperinsulinaemia induced by the glucose load that was the stimulus, rather than an increase in plasma glucose concentration.

In a study of 14 normotensive healthy young men, a two-step constant rate intravenous infusion of insulin, which achieved steady state insulin concentrations at

the upper end of the physiological range (~ 72 and ~ 144 mU/L), increased muscle nerve sympathetic activity, plasma noradrenaline level and heart rate, but there was no change in systolic blood pressure and a fall in diastolic blood pressure (Anderson et al 1991).

The most likely explanation for increased sympathetic activity induced by insulin is a central action, although a baroreceptor mechanism cannot be excluded. The fall in diastolic blood pressure in the latter study might be due to an unopposed sympathetic vasodilator mechanism, although a direct vasodilator action of insulin is possible and is discussed in the next section.

Lastly, there is evidence that insulin augments the pressor response to noradrenaline but not to angiotensin II (Gans et al 1991a). In this study 9 healthy males underwent a series of euglycaemic hyperinsulinaemic clamps (plasma insulin ~ 60 mU/L), and the pressor responses to noradrenaline and angiotensin II were investigated. During hyperinsulinaemia, the plasma concentration of noradrenaline required to raise diastolic blood pressure by 20 mm Hg was significantly reduced, whilst there was no effect upon the pressor dose of angiotensin II. This provides some evidence to support increased cardiovascular responsiveness to the sympathetic nervous system during hyperinsulinaemia.

1.2.3 HAEMODYNAMIC EFFECTS OF INSULIN

The potential effect of insulin to increase blood pressure via an effect upon the sympathetic nervous system was discussed in the preceding section, and an alternative pressor mechanism, the antinatriuretic action, will be discussed in

Section 1.2.4. Here I will discuss the vasodilator actions of insulin, which have recently been reviewed by Anderson (Anderson et al 1993).

In the previous section, I described how raising the plasma insulin concentration within the physiological range is associated with a rise in sympathetic activity but often with no increase in blood pressure (Rowe et al 1981; Berne et al 1989), or even a fall in blood pressure (Anderson et al 1991). It was only when plasma insulin was raised to supraphysiological levels that a rise in blood pressure was produced (Rowe et al 1981). Evidence from animal (Liang et al 1982) and human (Anderson et al 1991) studies have shown that the sympathetic vasoconstrictor activation produced by insulin is opposed by vasodilation. This provides an important mechanism by which insulin increases the supply of nutrients to peripheral muscle in the postprandial state. The mechanism by which this is achieved is unknown and there is evidence for both systemic and local dilator mechanisms.

Systemic mechanisms

Sympathetic neural vasodilation to the skin occurs in insulin-induced hypoglycaemia (Fagius et al 1989) and if this occurred in muscle it might provide an explanation. Mitigating against this, however, is the observation that insulin produces hypotension in patients with autonomic failure and presumed sympathetic denervation (Mathius et al 1987).

A second systemic mechanism might be release of a substance known to dilate the vascular bed in muscle. Adrenaline seems a likely candidate, but the

vasodilator response to insulin has been shown in the absence of an increase in circulating adrenaline (Liang et al 1982; Anderson et al 1991).

Local mechanisms

A number of potential local mechanisms have been suggested including: (i) β adrenergic mechanisms; (ii) endothelium dependent relaxation; (iii) stimulation of the sodium/potassium pump; (iv) increased CA^{2+} -ATPase activity; and (v) metabolic vasodilation.

(i) Studies both in animals (Liang et al 1982) and in humans (Creager et al 1985) have shown that the skeletal muscle vasodilator effects of insulin are abolished by pre-treatment with propranolol. In neither study did the insulin infusion cause an increase in circulating adrenaline. The production of local β adrenergic receptor stimulation in the absence of increased plasma adrenaline concentration raises the possibility that an increase in insulin might augment the β adrenoceptor response to adrenaline, as has been suggested for the α adrenoceptor response to adrenaline (Gans et al 1991a).

(ii) In vitro studies have suggested a relaxing effect of insulin in canine arteries (D'Orleans-Juste et al 1985), but this was present both in the presence and absence of endothelium. Although preliminary studies in rat and guinea-pig did not support insulin producing endothelium dependent vascular relaxation (Anderson et al 1993), a recent study in 9 healthy volunteers has suggested that the vasodilator effects of insulin in the forearm model are due to nitric oxide release (Scherrer et al 1994). In this study, using a euglycaemic clamp to increase insulin concentrations to ~ 70 mU/L it was possible to show that the effects of hyperinsulinaemia in increasing

forearm blood flow and decreasing forearm vascular resistance could be abolished by pre-treatment with N^G-mono-methyl-L-arginine (L-NMMA), a specific inhibitor of the synthesis of endothelium derived nitric oxide (NO). Indeed, administration of the glucose-insulin infusion was associated with a rise in blood pressure after pre-treatment with L-NMMA. However, despite these haemodynamic changes, there was no effect of treatment with L-NMMA on glucose uptake into muscle, indicating that this is independent of NO. The NO synthase enzyme is constitutively expressed in healthy endothelium and also exists in an inducible form in vascular smooth muscle cells. Expression of the inducible form is stimulated by inflammatory mediators such as interleukin-1 β and tumour necrosis factor- α which are released after vascular injury. In vitro work has shown that insulin-like growth factor 1, and to a lesser extent insulin itself, inhibit the release of NO from vascular smooth muscle cells that is stimulated by these mediators (Schini et al 1994). This suggests that insulin might differentially affect the production of NO by the constitutive and inducible forms of NO synthase amongst vascular cells. Consequently, the effect of insulin might differ from vasodilation in healthy vessels with intact endothelium to vasoconstriction in diseased vessels with exposed intima-media components. In support of this, recent data from subjects with vasospastic angina has suggested that fasting concentrations of serum insulin correlate positively with the degree of abnormal coronary vasoconstriction induced by intracoronary infusion of acetylcholine (Shimabukuro et al 1995).

(iii) The $\text{Na}^+\text{-K}^+$ pump and $\text{Na}^+\text{-K}^+\text{-ATPase}$ are stimulated by insulin in many tissues (Ferrannini et al 1988). In vascular smooth muscle, this can lead to hyperpolarisation and relaxation, thus causing vasodilation.

(iv) It has been suggested that insulin may stimulate the $\text{Ca}^{2+}\text{-ATPase}$ in the plasma membrane, leading to vascular relaxation and blunting of vasoconstrictor response (Zemel et al 1992).

(v) Insulin increases glycolytic flux and might cause metabolic vasodilation via net extrusion of protons or other metabolic intermediates. This was investigated in 12 healthy subjects, in whom local administration of insulin to the forearm had no haemodynamic action despite stimulation of glucose uptake and overall release of protons, lactate and pyruvate (Natali et al 1990). The plasma concentration of insulin attained in this study (~ 125 mU/L) was comparable to other studies (Anderson et al 1991) in which insulin was administered systemically and haemodynamic effects were observed. This argues against a local vasodilator action of insulin.

In summary, there is conflicting evidence for both systemic and local vasodilator actions of insulin. At present, the precise mechanisms remain obscure, but the observation that insulin induced vasodilation can be blocked by L-NMMA in man is most intriguing and points to endothelial release of nitric oxide as the final effector pathway (Scherrer et al 1994).

1.2.4 INSULIN EFFECTS UPON ELECTROLYTE BALANCE

In 1933, Atchley reported the metabolic effects of the abrupt withdrawal of insulin therapy from 2 diabetic patients. One of the most striking findings was a

marked increase in urinary sodium excretion which commenced within 24 hours and which reversed rapidly and completely on the resumption of therapy (Atchley et al 1933). The natriuresis commenced before hyperglycaemia and an osmotic diuresis developed, and this may partly account for the profound loss of salt and water often encountered in diabetic ketoacidosis.

De Fronzo demonstrated over 40 years later that an intravenous infusion of insulin to healthy volunteers (steady state insulin concentration ~ 150 mU/L), markedly reduced urinary sodium excretion whilst euglycaemia was maintained (De Fronzo et al 1975). The decrease in sodium excretion was accompanied by a fall in urinary potassium and phosphate, and by a rise in calcium excretion. These changes occurred in the absence of a change in glomerular filtration rate and renal plasma flow, and there was no change in plasma aldosterone. In a subsequent canine experiment, De Fronzo showed that insulin enhances sodium reabsorption in the distal tubule (De Fronzo et al 1976). The findings in humans were reproduced by Skott, who also showed that the effect of insulin was located distally to the proximal renal tubules, using lithium clearance as a measure of fluid output from the proximal tubule (Skott et al 1989). In a study using 3 ascending doses of insulin, Gans et al showed that the maximal reduction in fractional sodium excretion occurred within the normal physiological range of insulin concentrations, and that supraphysiological levels had no further effect (Gans et al 1991b).

It seems likely that the effect of insulin on sodium excretion in the distal tubule is mediated via stimulation of $\text{Na}^+\text{-K}^+\text{-ATPase}$ (Moore 1983), which is abundant in the distal tubule (Jorgensen 1980). In an elegant study, Ferrannini et al

demonstrated that the stimulation by insulin of $\text{Na}^+\text{-K}^+$ exchange in the human forearm is independent of the effects of insulin on the stimulation of glucose metabolism (Ferrannini et al 1988). In this study, an intra-arterial infusion of ouabain abolished the stimulatory effect of insulin on potassium uptake, by inhibition of the $\text{Na}^+\text{-K}^+$ pump, but had no significant effect on the rate of glucose uptake. This was a very important observation, because it hints at a compartmentation of the actions of insulin in vivo.

The influence of insulin on the renin-angiotensin-aldosterone system in humans might be important when considering the possible effects of angiotensin converting enzyme inhibition in hypertension or heart failure, but reports are inconclusive. At circulating insulin concentrations well within the normal physiological range (mean plasma insulin 66 mU/L) there was no effect upon plasma aldosterone and plasma renin (Skott et al 1989). In another study, at a mean plasma insulin concentration of 50 mU/L, there was a significant rise in plasma renin of ~50% compared to basal, but no change in aldosterone (Gans et al 1991b). A small study which used an insulin infusion for only 40 minutes to raise plasma insulin to 160 mU/L (above the normal physiological range), found a significant increase in plasma renin and angiotensin II, and a small fall in aldosterone (Trovati et al 1989). These effects, however, could be attributed to the rapid insulin-induced decrease in potassium encountered in this study, and they were abolished in a control study with concomitant potassium replacement. There is some evidence from animal studies that insulin may stimulate production of renin in the kidney and that this effect is diminished in streptozotocin-induced diabetes (Jost-Vu et al 1992).

Lastly, there is evidence that insulin stimulates renal Ca^{2+} -ATPase activity in several species (Levy et al 1989). Insulin also increases plasma membrane binding and phosphorylation of calmodulin, which is an important regulator of Ca^{2+} -ATPase activity (Levy et al 1989). There is, however, much less evidence from human studies to establish the importance of insulin in calcium metabolism, although in De Fronzo's study the infusion of insulin was associated with an increase in urinary calcium excretion (De Fronzo et al 1975).

1.2.5 EFFECTS OF INSULIN ON SPECIFIC CELL TYPES

The general metabolic effects of insulin have been described. With regard to arterial disease, 4 cell types are of particular importance, and the specific effects of insulin on these cell types will be discussed. These 4 include endothelial and smooth muscle cells from the arterial wall, and monocyte macrophages and platelets from the circulation.

Regulation of gene expression by insulin

After binding to its plasma membrane receptor, insulin can affect gene expression in the eukaryotic cell and this has recently been reviewed (Castano 1991). In general insulin seems to increase mRNA stability and its specific effect upon the transcription of several genes has been investigated. There is evidence for increased expression of some genes in the short term (minutes to 1 hour), and these include hepatic glucokinase and the viral proto-oncogene *c-myc*. There are longer term delays (1-24 hours) prior to increases in mRNA transcription for other genes, including lipoprotein lipase in adipocytes and fatty acid synthetase in 3T3 cells. In contrast,

insulin may reduce the transcription of certain mRNA including glucagon (short term) and growth hormone (long term). These findings may be of importance in the cellular proliferation which is characteristic of atheroma. Where there is information on the effect of insulin upon DNA turnover in the individual cell types involved in atheroma, it will be discussed below.

Endothelial cells

Human endothelial cells possess insulin receptors similar to those in other tissues (Bar et al 1978). The effects of insulin upon endothelial cells from bovine retinal artery and aorta were compared, and it was found that whilst insulin receptors were present on both, endothelium of aortic origin was not sensitive to insulin, whilst retinal artery endothelium responded with synthesis of glycogen and increased DNA production (King et al 1983). This may be relevant to the pathogenesis of diabetic retinopathy and in a broader sense might imply that the endothelium provides an insulin resistant protective layer in parts of the arterial tree. More recently it was shown that pathophysiological concentrations of insulin stimulate migration and tube-forming activity of bovine carotid artery endothelial cells (Nakao-Hayashi et al 1992). This might suggest a role in endothelial repair but also might be relevant to the neovascularisation which is a feature of the atherosclerotic plaque (Geiringer 1951).

Smooth muscle cells

Concentrations of insulin which are encountered in human subjects under physiological and pathophysiological conditions have been shown to cause proliferation of smooth muscle cells originating from primate (Stout et al 1975), rat

(Weinstein et al 1981), bovine (King et al 1983) and human (Pfeifle et al 1981) arteries. Although insulin receptors are present on the smooth muscle cell, there is strong evidence that the mitogenic effects of insulin are mediated via the receptor for insulin-like growth factor I (King et al 1980). Once again, this apparent support for a compartmentation of the actions of insulin is a very important observation. This leads to the suggestion that, in certain disease states, there may be defects in some, but not all, of insulin's actions, such that there might, for example, be an ineffective metabolic response but a normal proliferation response. It is also interesting that in the experiments mentioned above, the maximal growth promoting effects of insulin are often less than 50% those of bovine serum, which contains insulin-like growth factors possessing much greater affinity than insulin at their own receptor (King et al 1985; Bornfeldt et al 1991). An observation from a situation which may be analogous to insulin resistance being overcome by hyperinsulinaemia originated from a model of endothelial injury by balloon catheterisation in rats with streptozotocin-induced diabetes who showed an impaired vascular smooth muscle cell proliferative response to injury which could be overcome by chronic infusion of either insulin or insulin-like growth factor I (Bornfeldt et al 1992).

Insulin stimulates DNA synthesis by arterial smooth muscle cells, even in the absence of serum, and this may suggest that, in contrast to the endothelial cell, the smooth muscle cell is particularly sensitive to insulin (Taggart et al 1980). More recently, it has been shown that both insulin and insulin-like growth factor I cause an increase in the expression of the proto-oncogene *c-myc* in human arterial smooth muscle cells, although it was not clear which receptor was involved (Banskota et al

1989a). The same group showed that insulin, insulin-like growth factor I and platelet derived growth factor interact additively in the cellular proliferation and expression of *c-myc* in bovine smooth muscle cell culture (Banskota et al 1989b). This raises the intriguing possibility that insulin might be one of the agents that can modify the phenotypic expression of the smooth muscle cell between contractile and synthetic as reviewed recently by Ross (Ross 1993). However, some authors have suggested that acute effects of insulin in enhancing vascular smooth muscle cell proliferation may not persist in the presence of chronic stable hyperinsulinaemia (Ko et al 1993).

Two other effects of insulin may be of importance in the genesis of atheroma. Firstly, the migration of smooth muscle cells into the subendothelial space in order to release connective tissue proteins is central to their physiological role in repairing damaged endothelium and, if it becomes unregulated, to the formation of the atherosclerotic plaque. One of the chemoattractants released by activated platelets is 12-L-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE), and concentrations of insulin in the physiological range (25-100 m/UL) stimulate the 12-HETE induced smooth muscle cell migration in a manner dependent upon the prevailing glucose concentration (Nakao et al 1985). An interesting finding of this study was that the effect was only apparent when cells were pre-treated with insulin for several days and there was no effect with acute exposure to insulin. The combination of a relationship with glucose concentration and the need for long term incubation implies that both an energy-dependent pathway and the synthesis of macromolecules related to cell motility were necessary. A similar experiment using platelet derived growth factor as a chemoattractant did not find evidence for augmentation of

migration with insulin, but the lack of prolonged pre-exposure to insulin may be an explanation (Grotendorst et al 1982).

Secondly, the exposure to insulin of human skin fibroblasts, which are very similar to arterial smooth muscle cells, produces a downregulation of the number and affinity of high density lipoprotein (HDL) receptors and an upregulation of the low density lipoprotein (LDL) receptors (Oppenheimer et al 1989). In the same study it was possible to show that HDL mediated cholesterol efflux from cholesterol loaded cells was also significantly reduced. Thus, this study offered two mechanisms by which insulin may increase the accumulation of cholesterol within the arterial wall.

One last observation that may have clinical relevance is that the insulin-induced increases in RNA and protein synthesis in skeletal muscle cells are inhibited by indomethacin (Palmer et al 1989). This suggests that eicosanoid metabolites of arachidonic acid may play an important role in the long term control of myocyte growth, and that this may be influenced by inhibition of cyclo-oxygenase.

Monocyte macrophages

There has been less work examining the effects of insulin on monocytes with reference to arterial disease, although they are known to be rich in insulin receptors (Gavin et al 1973). The synthesis of cholesterol within the monocyte is increased by insulin via its effect upon the microsomal enzyme 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting step of sterol synthesis (Krone et al 1984). More importantly, the same group have shown that physiological concentrations of insulin stimulate the ability of monocytes to bind, accumulate and degrade LDL with high affinity, probably via an influence on LDL receptor numbers (Krone et al 1988).

Both of these observations may be relevant to the formation of foam cells from monocyte macrophages in the atherosclerotic lesion.

Apoptosis

Apoptosis, or programmed cell death, is manifest in most animal tissues and occurs when single cells die within otherwise healthy tissue (Wyllie 1993). There are associated histological changes which are similar in different tissues, but the molecular mechanisms involved are not known. Apoptosis differs fundamentally from necrosis, the pathological form of cell death in which the cell swells and lyses, because it is consistently characterised by cell shrinkage, condensation of cytoplasmic and nuclear components, cleavage of chromosomal DNA into fragments (~200 base pairs in length), and enhanced recognition of the cell by phagocytes. There is increasing evidence that most animal cells contain cell suicide programmes which may be activated or suppressed by signals from other cells, and that this represents an anti-proliferative response. The most extreme view is that all cells would undergo apoptosis in the absence of signals telling them not to (Raff 1992). As discussed above, when fibroblasts are stimulated to proliferate in response to growth factors, one response is increased expression of the proto-oncogene *c-myc*. There is evidence that the cellular response to *c-myc* is critically dependent upon the availability of certain growth factors, which may determine either proliferation or apoptosis (Evan 1992). The most influential growth factor for fibroblasts seems to be insulin-like growth factor I and it has been shown that insulin and insulin-like growth factor I prolong survival, or inhibit apoptosis, in oligodendrocytes (Barres et al 1992). This raises the possibility that these agents are not proliferative factors, but are

survival factors. Human umbilical vein endothelial cells undergo apoptosis if either fibroblast growth factor or bovine serum albumin, which contains insulin-like growth factor (Svrzic et al 1990), are removed from their medium (Araki et al 1990). Isolated monocytes undergo apoptosis in the absence of exogenous stimuli (Mangan et al 1991) and this may be inhibited by cell adherence, microbial products, and some cytokines, but is not inhibited by chemoattractants or other cytokines including insulin-like growth factors (Mangan et al 1993).

Most interest in apoptosis has centred on its possible role in carcinogenesis, although the possibility of an influence in chronic inflammatory disease has recently been reviewed (Mangan et al 1993). A novel suggestion, if entirely speculative, would be that a possible role for insulin in atherogenesis is in acting as a survival factor for the cells involved in the “proliferation” of the atherosclerotic plaque by preventing or delaying programmed cell death.

Platelets

Human platelets have been shown to possess insulin receptors (Hajek et al 1979), although the biological role of these receptors is largely unknown. Trovati et al investigated the aggregatory response of platelets after incubation of platelet rich plasma from normal volunteers with increasing doses of insulin for 5 or 30 minutes. They observed a significant reduction of platelet sensitivity to all the aggregating agents employed, including ADP, platelet-activating factor, adrenaline, collagen, and arachidonate. This effect occurred at physiological concentrations of insulin (~40 mU/L), was reversible, and was time and dose-dependent (Trovati et al 1988). Kahn et al showed that insulin increased binding of prostaglandin E_1 at the platelet

prostaglandin E_1/I_2 receptor in platelet rich plasma from normal subjects, and that it restored prostaglandin E_1 binding to normal level in platelets from 75% of patients with acute coronary syndromes (Kahn et al 1991). This group found that the platelets from 25% of patients with acute coronary syndromes appeared to be resistant to this action of insulin. In a further study of patients suffering unstable angina or acute myocardial infarction, they showed that intravenous boli of insulin increased circulating levels of prostacyclin and decreased the minimum inhibitory concentration of prostaglandin E_1 for platelet aggregation to ADP. However, this effect appeared to be abolished by the concurrent administration of aspirin (Kahn et al 1992). Lastly, physiological concentrations of insulin increase intracellular magnesium transport in human platelets, but have no effect upon calcium concentrations (Hwang et al 1993). In the same study, insulin was shown to diminish platelet aggregation to thrombin and to reduce production of thromboxane B_2 and 12-HETE. These effects were abolished by a specific anti-insulin receptor monoclonal antibody.

Thus, there is some evidence that concentrations of insulin in the postprandial range inhibit platelet aggregation, although the teliological significance of this is not clear. This effect is not present in some individuals with ischaemic heart disease and, in addition, it may be blocked by administration of aspirin.

1.3 EFFECTS OF INSULIN ON ARTERIES

Duff et al reported in 1954 that the failure of atheroma to develop in alloxan-treated, cholesterol-fed rats was corrected when their insulin-deficient diabetic state was treated with insulin replacement (Duff et al 1954). In a beautifully designed study, Cruz et al observed in alloxan-treated diabetic dogs that the infusion of insulin into the right femoral artery, compared to saline in the left femoral artery, was associated with a significant increase in the cholesterol content of the artery (Cruz et al 1961). In a series of studies, Stout showed that acute administration of insulin stimulated lipogenesis and cholesterol synthesis in rat aorta, and then that chronic treatment of chickens with insulin caused the development of atheromatous lesions in the aorta (Stout 1968, 1969 and 1970). Almost 20 years later, Sato et al reported that Wistar rats who were rendered hyperinsulinaemic by daily injections of insulin for one year, developed atheromatous lesions in the aorta (Sato et al 1989). In particular, the triglyceride content of the aorta was increased, and, at light microscopy, the aortic intima of the insulin-treated rats, compared to saline-treated rats, was significantly thickened and contained eosinophilic fibre bundles, amorphous ground substances, and irregularly arranged cells. Electron microscopy confirmed that these cells were of smooth muscle origin. Fasting concentrations of insulin were similar in the 2 groups (27 v 26 mU/L) but the insulin injection caused an supraphysiological peak (616 v 44 mU/L) and approximately 12 hours of hyperinsulinaemia. Despite this, there was no effect upon plasma total cholesterol, triglyceride, phospholipid or non-esterified fatty acids. Thus, this study supported the earlier work suggesting a

role for insulin in the development of atheroma independent of any effect upon circulating lipoproteins, and also demonstrated that the smooth muscle cell was evident in these lesions.

Insight into the metabolic mechanisms that might be involved in these changes was provided by a study in which a chronic hyperinsulinaemic state associated with impaired glucose tolerance was induced in pigs by stimulating production of antibodies to insulin (Falholt et al 1985a). In this study, the triglyceride content of the aorta and of peripheral muscle was increased 10-15 fold compared to control animals. Enzyme analysis showed increased activity of hydroxyacyl-CoA-dehydrogenase, suggesting increased fatty acid oxidation, and higher concentrations of glucose-6-phosphate dehydrogenase, possibly related indirectly to increased fatty acid synthesis via a provision of NADPH. In general, the activity of enzymes involved in glycolysis was decreased and there was evidence that the metabolic derangement was more marked in the aorta than in muscle. Further studies from Falholt et al using a canine model of chronic hyperinsulinaemia supported these findings (Falholt et al 1985b).

Evidence for an influence of insulin on human arteries has recently been provided from the ARIC study in which the mean thickness of the intima-media of the carotid artery was measured in 7956 North American subjects using B-mode ultrasound (Folsom et al 1994). In this study, fasting insulin showed a positive correlation with artery wall thickness, with an estimated increase of 0.02 mm for an increase of 14 mU/L plasma insulin. Unfortunately, no measure of stimulated insulin response to glucose, or of insulin sensitivity, was made in this study.

Not all studies have supported a role for insulin in the development of atheroma. In general, the evidence suggests that it is chronic exposure to insulin which leads to changes in the arterial wall. In one study, a single dose of insulin failed to increase uptake of cholesterol into the aorta of rabbits (Christensen et al 1965). A more recent study of the acute proliferative response of the rat aorta to balloon injury showed that exposure to increased insulin for 2-4 days after the injury did not influence the acute proliferation response of the intima-media smooth muscle cell (Ridray et al 1992).

1.4 PHYSIOLOGICAL EFFECTS OF INSULIN ON ENDOGENOUS FIBRINOLYSIS

The ability of the blood to lyse intravascular thrombus using plasmin is principally controlled by the balance between plasminogen activators and plasminogen activator inhibitors. The observation that a positive correlation existed in normal subjects between fasting insulin and plasminogen activator inhibitor type I (PAI-1), the most physiologically important of the inhibitors (Vague et al 1986), led to a number of studies which have attempted to explore the relationship between these two.

In vitro studies

PAI-1 is synthesised in hepatocytes and endothelial cells in culture, and is found in high concentrations in platelets. The effect of insulin on PAI-1 synthesis was first studied in a comparison of hepatocytes from a human hepatocellular

carcinoma cell line (Hep G2) and endothelial cells from human umbilical vein (Alessi et al 1988). Using a range of insulin concentrations that remained within physiological limits, Alessi et al showed a dose dependent increase of PAI-1 antigen and activity in the culture medium of the hepatocytes, but there was no increase in the endothelial cell culture. Using the same method they found no effect of human proinsulin in either cell culture. The effect of insulin could be blocked by cycloheximide and actinomycin D, indicating that it depended upon de novo protein and RNA synthesis. Total protein synthesis was similar before and after stimulation of the hepatocytes with insulin, suggesting that the increase in PAI-1 was a relatively specific effect. Kooistra et al confirmed that insulin stimulated PAI-1 synthesis in human hepatocytes using cells obtained from a liver transplant programme and they showed that the increase in PAI-1 could be entirely explained by a concomitant increase in PAI-1 mRNA levels (Kooistra et al 1989). A later study found no influence of insulin on the production of tissue-type plasminogen activator (t-PA) in hepatocyte culture (Seki et al 1990). More recently Anfosso et al showed that the stimulating effect of insulin on PAI-1 synthesis by Hep G2 cells persisted even when insulin receptor numbers were downregulated by prolonged exposure to insulin (Anfosso et al 1993). In the same study, the stimulating effect of insulin on PAI-1 was inhibited by metformin, and although the exact mechanism of this is unknown, it is in contrast to metformin's action in increasing the biological effect of insulin on metabolism.

In vivo studies

Attempts to show an acute influence of insulin on fibrinolysis in non-diabetic humans have been much less revealing than the *in vitro* studies - probably because of their short time scale and the difficulty in increasing circulating insulin concentrations over a prolonged period in non-diabetic subjects.

Medvescek et al measured the PAI-1 response to a high calorie carbohydrate meal in 10 lean and 11 obese individuals and suggested that there might be a transient rise in PAI-1 antigen and activity at 1 hour, when insulin levels were at a peak, and that this was superimposed upon the normal diurnal fall of PAI-1 seen over the morning hours (Medvescek et al 1990). However, intravenous infusions of insulin with glucose during a hyperinsulinaemic euglycaemic clamp in normal subjects did not cause a rise in PAI-1 over 120 minutes (Grant et al 1990a), 6 hours (Potter van Loon et al 1990) or over 3 hours (Vuorinen-Markkola et al 1992). One study measured the response of both t-PA and PAI-1 to an intravenous glucose and insulin infusion in 9 normal subjects and again failed to show any continuing alteration of normal diurnal shift over a 2 hour period (Landin et al 1991).

Thus, whilst *in vitro* studies support a possible role for insulin in the regulation of hepatic production of PAI-1, it is much more difficult to show an *in vivo* effect in short term studies. Some indirect evidence is provided from studies in which dietary changes to reduce calorie intake (Sundell et al 1989) or increasing exercise (Williams et al 1980), both of which tend to reduce insulin levels, were associated with improved fibrinolytic function, but actual insulin concentrations were not measured in these studies.

1.5 INSULIN IN ISCHAEMIC HEART DISEASE

The evidence for a role of insulin in patients with ischaemic heart disease can be broadly divided into studies comparing populations with different levels of risk, prospective longitudinal studies which have identified elevated insulin concentrations to be a risk factor for developing ischaemic heart disease, and cross-sectional clinical studies showing elevated concentrations of insulin to be a feature of patients with established ischaemic heart disease.

1.5.1 POPULATION STUDIES

Epidemiological studies have shown that different populations have different levels of risk for developing ischaemic heart disease. One approach to identifying potential risk factors is to compare them in populations at different levels of risk and extrapolate from this their possible influence. A few studies have used this approach for insulin, but the Edinburgh-Stockholm study deserves special mention as it was performed on our own population and because it was one of the major stimuli to undertaking this thesis (Logan et al 1978). Twenty years ago the mortality from ischaemic heart disease for 40 year old men was three times greater in Edinburgh than in Stockholm. A study was designed to compare the prevalence of classical and some potential new metabolic risk factors in matched cohorts of normal 40 year old men from the two cities. Over a 6 month period, 107 Edinburgh men and 82 Stockholm men were studied. Edinburgh men were shorter, more obese, had higher

blood pressure, smoked more cigarettes, drank more alcohol, had more resting electrocardiographic abnormalities and lower exercise tolerance. Edinburgh men had a more adverse lipid profile, lower levels of plasma and adipose tissue linoleic acid and a lower polyunsaturated/saturated fatty acid ratio. However, and most importantly for this thesis, in response to an oral glucose tolerance test, *for an identical glucose response, Edinburgh men had a much greater insulin response.* Although it was recognised that this implied insulin resistance, and this was 10 years before Reaven's landmark lecture (Reaven 1988), amongst all the other findings of the study, this one did not receive a great deal of attention.

A 12 year follow up of the Edinburgh cohort was undertaken and 11 men were found to have suffered a cardiovascular event, which included the development of angina, myocardial infarction, coronary artery surgery or sudden cardiac death (Hargreaves et al 1992). There was no significant difference in baseline insulinogenic indices between the men who developed one of the endpoints and men who remained free of cardiovascular events. However, only 4 men suffered myocardial infarction in this group (RA Riemersma, personal communication) and this makes it difficult to draw any firm conclusion about insulin as a risk factor for myocardial infarction within the Edinburgh population.

Four studies have sought abnormalities of insulin response in populations of Asian origin within the United Kingdom and compared with subjects of European ancestry. McKeigue et al described stimulated hyperinsulinaemia in 253 Bangladeshi men and women living in London but they did not measure fasting insulin (McKeigue et al 1988). In a subsequent and much larger study, both fasting and

stimulated hyperinsulinaemia were a feature of 1421 South Asian men, and these were also associated with electrocardiographic evidence of premature ischaemic heart disease (McKeigue et al 1993). Stimulated hyperinsulinaemia was confirmed in a different population of Asian men living in Bradford (Knight et al 1992). In a study of patients surviving myocardial infarction, Hughes et al compared the 2 hour insulin response in British Asian and white men and found even greater hyperinsulinaemia in the Asian survivors (Hughes et al 1989).

1.5.2 PROSPECTIVE STUDIES

In the Helsinki Policemen Study, 982 men aged 35-64 years underwent a baseline oral glucose tolerance test (glucose load 75 g or 90 g dependent on body surface area) with measurement of plasma insulin at baseline fasting, 1 and 2 hours. The major endpoints were fatal and non-fatal myocardial infarction, and after 5 years these were more common in those with the highest insulin concentrations at each of the above time points (Pyorala 1979). A further follow up after 9½ years showed that these relative risks had persisted (Pyorala et al 1985). Other classical risk factors that were also measured included blood pressure, total cholesterol and triglyceride levels, smoking, body mass index, physical activity score and blood glucose. When these factors were included in multivariate analysis, a significant independent excess risk for those in the highest decile for 60 and 120 minutes insulin values persisted, although the adjusted relative risk for fasting plasma insulin was not significant ($p = 0.09$). One interesting finding of this study in view of a recent suggestion that cigarette smoking is associated with hyperinsulinaemia and insulin resistance

(Facchini et al 1992) was that those who were current smokers (n = 426) had similar fasting plasma insulin concentrations and significantly *lower* 2 hour insulin values when compared with non-smokers.

In the Paris Prospective Study, 7246 men aged 43-54 years underwent a baseline 75 g oral glucose tolerance test with measurement of plasma insulin concentrations at fasting baseline and at 2 hours. The major endpoints were fatal and non-fatal myocardial infarction, and after 5 years these were more common in those with the highest insulin values (Ducimetiere et al 1980). In multivariate analysis which also included total cholesterol, systolic blood pressure, cigarette consumption and plasma glucose, only fasting plasma insulin concentration remained a significant independent predictor of risk.

In the Busselton, Western Australia Study, 3390 men and women representing 91% of the adult population of Busselton underwent a non-fasting 50 g oral glucose tolerance test and a single blood sample was taken at 60 minutes. The major endpoints were the 6 year incidence of ischaemic heart disease, including stable angina, and the 12 year mortality from ischaemic heart disease. Men in the highest quintile for plasma insulin were at significantly greater risk for all major endpoints, but there was no apparent risk associated with hyperinsulinaemia in women (Welborn et al 1979). A subsequent multivariate analysis of the 13 years mortality in a subgroup of 1654 subjects from this study suggested that total mortality in men aged 60-74 years was positively related to the 60 minute insulin level, but that total mortality was negatively related to insulin in men aged 40-59 years (Cullen et al 1983).

Thus, although these studies provide support for an aetiological role of insulin in the development of ischaemic heart disease in men, they do not present a consistent picture, with emphasis not resting clearly on either fasting insulin concentrations or the stimulated insulin response to an oral glucose load. One problem is that the inter-relationships which exist between insulin, blood pressure, plasma lipids, body mass index, and possibly cigarette smoking, make the interpretation of multivariate analysis suspect. As with many risk factors for ischaemic heart disease, the importance of insulin for women is less clear, but what evidence there is, recently reviewed by Fontbonne, suggests that hyperinsulinaemia may not be a risk factor for ischaemic heart disease in women (Fontbonne 1991).

1.5.3 CROSS-SECTIONAL STUDIES

The initial report that plasma insulin concentrations were elevated in patients with ischaemic heart disease was made by Peters and Hales in 1965. Seven patients underwent an oral glucose tolerance test at a mean of 12 months from the time of myocardial infarction and were found to have significant fasting and stimulated hyperinsulinaemia when compared to controls. Since then there have been a number of studies examining the glucose and insulin response to an oral glucose load and some details of these are given in Table 1. Important factors to consider in the interpretation of this data include:

- (1) Sample size - from 7 patients (Peters and Hales 1965) to 499 patients (Lichtenstein et al 1987, fasting blood sample only).

(2) Many studies have exclusively included patients with prior myocardial infarction, often at different times in their convalescence, and others have also included patients with stable angina. No study has included an assessment of ventricular function.

(3) Most studies have excluded diabetics but many have included patients with abnormal or impaired glucose tolerance.

(4) Most studies have not excluded patients with a previous history of hypertension.

Other than the sample size, the above considerations are important because they may affect the expression of hyperinsulinaemia. The pathogenesis of episodes of chronic stable angina and myocardial infarction are different. Whereas the presence of atheroma is important for both, acute myocardial infarction develops as a consequence of acute occlusive intracoronary thrombus, although episodes of haemorrhage into a fissured plaque without luminal obstruction may contribute to the progression of atheroma (Fuster et al 1992a, 1992b). The preceding discussion of the actions of insulin has shown how it may influence either atheroma progression or risk of thrombosis. The potential confounding effect upon hyperinsulinaemia of impaired ventricular function and heart failure has not been addressed in any of these studies. The neurohumoral response to chronic ventricular impairment (Bayliss J et al 1987) which leads to increased secretion of catecholamines, cortisol and growth hormone might have an important influence in antagonising the effects of insulin (Brindley and Rolland 1989).

Three conditions which are more prevalent amongst patients with ischaemic heart disease than in the general population are hypertension, impaired glucose tolerance and non-insulin dependent diabetes mellitus. All of these are associated with hyperinsulinaemia and peripheral insulin resistance (Reaven 1988). Many previous studies have included patients with these conditions and have failed to address their potentially confounding influence.

Table 1 gives an overview of the published work and reveals that the most consistent feature of patients with ischaemic heart disease is stimulated hyperinsulinaemia in response to an oral glucose load. Three studies did not find post-load hyperinsulinaemia. In one study which included patients without prior myocardial infarction, insulin concentrations were lower in those with only angina, and stimulated hyperinsulinaemia was only present in those with impaired glucose tolerance (Gertler et al 1972). The study of Bergstrand et al examined male survivors of myocardial infarction, but who were studied at a mean of 3 years from the time of infarction, and who were matched by age and plasma lipids to controls (Bergstrand et al 1979). Both the long delay from the time of infarction and the matching for plasma lipids, which are often abnormal in the hyperinsulinaemia syndrome, may account for the absence of a difference in insulin response between patients and controls. Lastly, Jackson et al studied 7 men with myocardial infarction under the age of 40 years and did not find hyperinsulinaemia to be a feature (Jackson et al 1983). Despite the small size of this study, and in conjunction with the previous study, this might suggest that hyperinsulinaemia was not important in young people with ischaemic heart disease. However, this hypothesis is refuted by the larger series of Anders Hamsten, which

did find hyperinsulinaemia in 104 male survivors of myocardial infarction aged less than 45 years (Hamsten et al 1987).

Three studies have described fasting hyperinsulinaemia in patients with ischaemic heart disease. The studies of Peters (Peters and Hales 1965) and Larsen (Larsen et al 1981) included only 7 and 10 subjects respectively. However, the very large Caerphilly study suggested that fasting plasma insulin concentrations were 29% higher amongst 499 patients with ischaemic heart disease compared to 1526 controls, and because of its size this study had by far the greatest power of any to detect such a difference (Lichtenstein et al 1987).

In summary, previous studies suggest that stimulated post-glucose load hyperinsulinaemia is a feature of patients with ischaemic heart disease and possibly fasting hyperinsulinaemia also. Analysis of these studies is hampered by heterogeneous patient groups, the timing of studies with relation to myocardial infarction, and failure to exclude subjects with other important conditions associated with hyperinsulinaemia.

1.5.4 HYPERINSULINAEMIA AND THE METABOLIC SYNDROME

In trying to establish the significance of hyperinsulinaemia in the pathogenesis of ischaemic heart disease it is difficult to dissociate the links between insulin and other individual coronary risk factors. In particular a clustering of several of these risk factors occurs in many individuals (Zavaroni et al 1987 and 1989). The 1988 Banting Lecture from Gerald Reaven was a very good introduction to the possible role of insulin and this risk factor syndrome in human disease

(Reaven 1988), and De Fronzo and Ferrannini have produced a more recent review of the mechanisms which may be involved (De Fronzo and Ferrannini 1991). Hyperinsulinaemia has been associated with hypertension, dyslipidaemia, upper-body obesity, decreased physical activity, impaired glucose tolerance and non-insulin dependent diabetes mellitus.

Young non-obese subjects with untreated moderately severe essential hypertension have been shown to have stimulated hyperinsulinaemia and peripheral insulin resistance (Ferrannini et al 1987). Most studies have described a positive relationship between the degree of hyperinsulinaemia and blood pressure in hypertensives, but several have not found such a relationship in normotensives. The chicken and egg argument is difficult to resolve but possible aetiological mechanisms for insulin include sodium retention at the kidney, effects on autonomic balance, and direct haemodynamic effects.

In large population-based studies, hyperinsulinaemia has been associated with a dyslipidaemic profile including raised levels of plasma very low density lipoprotein (VLDL) cholesterol and triglyceride, raised levels of low density lipoprotein (LDL) cholesterol and triglyceride, and low levels of high density lipoprotein HDL) cholesterol (Modan et al 1988). Whilst the relationship between insulin, VLDL and HDL cholesterol is strikingly consistent, there are discrepancies for the relationship with total and LDL cholesterol. A recent study has suggested that individuals who are hypercholesterolaemic but who have normal triglyceride levels do not have abnormalities of glucose and insulin metabolism (Sheu et al 1993). The likely mechanism by which insulin influences lipid profile is by stimulating hepatic VLDL



synthesis, which would explain the elevated total triglycerides, but the exact mechanism for the reduction in HDL levels remains unclear.

It was assumed for many years that patients with impaired glucose tolerance or non-insulin dependent diabetes mellitus were suffering from an absolute deficiency of insulin. It is now clear that for the vast majority with impaired glucose tolerance, and for those with non-insulin dependent diabetes mellitus who have not reached an advanced stage of the disease, absolute concentrations of immunoreactive insulin are greater than in normal controls both for fasting and stimulated, and that this is due to the presence of peripheral insulin resistance (Reaven et al 1989). Recently it has been suggested that conventional insulin assays may overestimate the concentrations of insulin because of cross-reactivity with abnormally elevated levels of proinsulin and proinsulin split products in non-insulin dependent diabetes (Temple et al 1990). However, in subjects with diet-controlled diabetes (Clark et al 1992) and in impaired glucose tolerance (Davies et al 1993) these do not seem to comprise a significant proportion of the total.

Several groups have proposed a clustering of risk factors including hypertension, dyslipidaemia, impaired glucose tolerance, obesity, hyperinsulinaemia and insulin resistance. These have been referred to as the GOH conditions (Modan et al 1985), Syndrome X (Reaven 1988) or the metabolic cardiovascular syndrome (Hjermann 1992).

This metabolic syndrome was well illustrated in a study in which 32 healthy, normotensive, non-obese subjects were identified as having hyperinsulinaemia on the basis of having a fasting or post-glucose load insulin more than 2 standard deviations

higher than the mean of a total group of 247 volunteers, all with normal glucose tolerance (Zavaroni et al 1989). The hyperinsulinaemic group were matched for age, sex and body mass index with 32 controls and were shown to have higher plasma triglyceride levels, lower HDL cholesterol levels, higher systolic and diastolic blood pressures, and higher post-load plasma glucose levels.

1.6 HYPERINSULINAEMIA AND INSULIN RESISTANCE

The terms hyperinsulinaemia and insulin resistance are often used interchangeably but whilst the two conditions frequently coexist, the terms have different meanings. Hyperinsulinaemia implies either abnormally elevated fasting concentrations of insulin, or an increased insulin response to a stimulus - usually an orally or intravenously administered glucose load. Insulin resistance describes a state in which the biological effect of insulin on glucose metabolism is less than it should be, and this usually leads to a chronic compensatory hyperinsulinaemia. However, the ability of the pancreas to maintain this response may wane as islet cells become exhausted and thus lead to the combination of insulin resistance with low circulating levels of insulin. Such changes are thought to occur in many individuals as they develop non-insulin dependent diabetes mellitus and glycaemic control deteriorates (Saad et al 1989). The biological defect in most insulin resistant states is fairly tissue specific (mostly involving skeletal muscle), selective (mostly involving insulin-stimulated glucose uptake), pathway specific (mostly involving non-oxidative pathways e.g. glycogen metabolism), and partial (i.e. a reduction in function but not

a complete loss). However, some differences in the expression of insulin resistance according to the clinical presentation are shown in Table 2. To what extent the defects in insulin action extend to effects of insulin other than on glucose, such as potassium balance, has not been clearly defined as yet. There is some preliminary evidence that in obesity and in hypertension, insulin-mediated vasodilation may also be impaired (Feldman and Bierbrier 1993).

In this thesis I do not use the terms hyperinsulinaemia and insulin resistance interchangeably, and when insulin resistance is used it implies that a method of quantifying insulin resistance has been used. The most widely accepted and “gold standard” technique is the euglycaemic hyperinsulinaemic clamp described by De Fronzo (De Fronzo et al 1979), its major advantage being that measurements are made at steady state concentrations of glucose and insulin. A number of other methodologies have been described, and are briefly reviewed in Chapter 2, but none have usurped the primacy of the “clamp.” The euglycaemic clamp has been used to quantify insulin resistance in subjects with obesity, impaired glucose tolerance, insulin dependent and non-insulin dependent diabetes mellitus, and essential hypertension (Reaven 1988; De Fronzo and Ferrannini 1991). Whilst it has been inferred that patients with ischaemic heart disease are insulin resistant, no studies as yet have employed the euglycaemic clamp to evaluate this. Recently, two studies have used alternative methods to suggest the presence of insulin resistance. Young et al used the insulin suppression test in 20 patients with angiographically verified coronary artery disease to show insulin resistance compared to controls, but failed to exclude patients with impaired glucose tolerance and did not describe the presence of

previous infarction or heart failure (Young et al 1993). Ley et al used a frequently sampled intravenous glucose tolerance test combined with a mathematical model to show insulin resistance in 37 non-obese men with angina and abnormal coronary angiograms (Ley et al 1994). The complexity and time-consuming nature of the euglycaemic clamp preclude its use in large scale clinical studies, but it nevertheless remains the least controversial of the different methods for measuring insulin sensitivity.

1.7 EFFECTS OF DRUG THERAPY ON HYPERINSULINAEMIA AND INSULIN RESISTANCE

The vast majority of work investigating the pharmacological manipulation of insulin responses has been carried out in subjects with either hypertension or diabetes mellitus or both. Very few studies have addressed the effects of commonly prescribed drugs in patients with ischaemic heart disease, and for compounds including β adrenoceptor antagonists, calcium antagonists, angiotensin converting enzymes (ACE) inhibitors, and diuretics, it tends to be assumed that their effects would be the same in hypertension and ischaemic heart disease. The possible effects on insulin resistance of presently available and new agents which are under development was recently reviewed by Donnelly and Morris (Donnelly and Morris 1994).

The effect of β blockers on glucose tolerance and insulin response in patients with ischaemic heart disease was studied in a comparison of the effects of

propranolol and acebutolol (Birnbaum et al 1983). There was no change in insulin secretion in this study. Other chronic studies have employed only hypertensive subjects and no clear effect has been demonstrated (Ekberg et al 1977; Day et al 1979; Berglund et al 1981; Lehtonen 1984; Cressman et al 1985). One study, which used the euglycaemic clamp to measure insulin sensitivity, suggested that chronic administration of atenolol and metoprolol was associated with a decrease in insulin sensitivity (Pollare et al 1989a). However, this study is difficult to interpret because of a significant weight gain in both groups over the study period. In a recent review it was concluded that there is no evidence for an adverse effect of β_1 -selective β blockers on glucose and insulin parameters (Sawicki and Berger 1992).

There is no compelling evidence for an adverse metabolic effect of calcium antagonists, and in particular no support for an influence of these agents on glucose and insulin responses, despite an extensive literature which has been reviewed by Trost (Trost 1987).

An overview of studies which have explored the possible influence of ACE inhibitors on hyperinsulinaemia and insulin resistance appears in Table 3. Although a large number of such studies have been reported in the last few years, it is striking how few have employed adequate experimental design with, for example, double-blind conditions and placebo control. One of the earliest and most influential reports appeared from Pollare et al and compared the effect of captopril and hydrochlorothiazide in 50 hypertensive subjects who underwent metabolic investigations after a placebo run-in period, after 4 months treatment with one drug followed by a 4 week washout phase and then 4 months treatment with the other drug

(Pollare et al 1989b). Unfortunately, analysis was hampered by a hangover effect of thiazide treatment and therefore results were presented as a parallel group study of the first treatment period only. There was no hangover effect with captopril. Captopril was shown to increase glucose disposal, the measure of insulin sensitivity, from 5.7 to 6.3 mg/kg/min ($p < 0.05$), whereas hydrochlorothiazide caused a decrease from 6.4 to 5.7 mg/kg/min ($p < 0.01$). Captopril had no effect upon fasting glucose, insulin or lipoproteins, but did cause a significant decrease in the stimulated insulin response to an intravenous glucose load. This group recently reported a follow up after 2-3 years treatment with captopril in a minority subgroup of the original cohort and found that the initial improvement in insulin sensitivity appeared to be maintained, although differences between baseline and 2 years were no longer statistically significant (Lind et al 1994).

Since the original study there have been many others which have looked at normal subjects, hypertensives, diabetics, and hypertensive diabetics, and which have investigated a variety of ACE inhibitors. No consistent picture has emerged, with the strongest evidence of an effect being for captopril. No study has found an effect on fasting insulin and only two have suggested a fall in stimulated hyperinsulinaemia. Four studies using the euglycaemic clamp have reported an increase in insulin sensitivity, three after treatment with captopril and one after enalapril, and with durations of treatment ranging from a single dose to 4 months. Eight studies of various ACE inhibitors using a variety of methods to measure insulin sensitivity have found no effect. One recent study which included both animal and clinical experiments and which compared an ACE inhibitor with a sulphydryl group

(captopril) to two without (enalapril and delapril), found that only captopril was associated with an increase in insulin sensitivity (Uehara et al 1994). Also in this study, only captopril was associated with an increase in plasma bradykinin; and infusion of a bradykinin antagonist abolished the effect of captopril on insulin sensitivity. This lead to the conclusion that ACE inhibitors with a sulphydryl group may have the most potent effects on insulin action and that one possible mechanism is via an effect on bradykinin. This view is supported by earlier evidence that captopril enhanced forearm uptake of glucose in diabetic patients in association with a rise in kinin levels (Jauch et al 1987). The same group later showed that in post operative subjects an infusion of bradykinin increased glucose disposal (Hartl et al 1990).

Other mechanisms by which ACE inhibitors might enhance insulin action include the inhibition of the formation of angiotensin II and their vasodilator action. As discussed earlier (Section 1.2.4), there is no strong evidence to show an effect of insulin upon the renin-angiotensin system, and likewise there is little evidence for a direct effect of components of the renin-angiotensin system at physiological concentrations on insulin action (Morris et al 1993). There is much stronger evidence that skeletal muscle blood flow is important in determining muscle glucose uptake (Ganrot 1993), which is one of the main defects in the insulin resistance syndrome, and thus improved muscle blood flow secondary to ACE inhibition might explain improved insulin action. Furthermore, one study has shown that prazosin, another vasodilator, improves insulin sensitivity in hypertensive subjects (Pollare et al 1988).

Two recent studies which infused pressor doses of angiotensin II (Buchanan et al 1993; Townsend et al 1993) to normal volunteers showed an increase in insulin-mediated glucose disposal, and in patients with Type 2 diet-controlled diabetes mellitus Morris found that both subpressor and pressor doses of angiotensin II improved glucose uptake (Morris et al 1994). It remains unclear as to whether these effects can be wholly accounted for by alterations in muscle blood flow caused by the haemodynamic effects of angiotensin II, or whether it may perhaps have some unsuspected metabolic effect, but the former seems most likely.

It is widely known that the thiazide diuretics are associated with a deterioration in glucose tolerance which may sometimes be clinically important and Pollare et al confirmed that hydrochlorothiazide caused a decrease in insulin sensitivity as discussed above (Pollare et al 1989b). These drugs tend to be used in the management of hypertension, but in normotensive patients with ischaemic heart disease the need for diuretics arises when cardiac failure develops and in this situation loop diuretics are usually employed. Although there are case reports of these being associated with deterioration of glucose tolerance (Toivenen and Mustala 1966), in studies which examined their effect upon glucose tolerance and insulin response when given for hypertension (Jackson and Nellen 1966) or for heart failure after myocardial infarction (Efendic et al 1984) there was no evidence of an effect on hyperinsulinaemia.

1.8 ENDOGENOUS FIBRINOLYSIS AND THE RISK OF THROMBOSIS

After a prolonged period of controversy, it is now accepted that the cause of acute myocardial infarction in the overwhelming majority of cases is the formation of occlusive thrombus within a coronary artery (De Wood et al 1980). Thrombosis results from the deposition of abnormal quantities of fibrin and platelets within the vessel lumen. Initially platelets adhere to the vessel wall, a further platelet aggregation takes place, and then the platelets discharge their contents locally. The crucial point after activation of the coagulation cascade from either the intrinsic or extrinsic pathway is the development of thrombin. Thrombin stimulates the formation of soluble fibrin monomer from circulating fibrinogen, and thereafter insoluble fibrin polymer precipitates and fibrin crosslinking takes place. Platelets, red blood cells and other circulating components are adsorbed onto the thrombus and it becomes established. However, a system exists which, in the basal state, is equal and opposite in action to the forces of coagulation, the fibrinolytic system. This system ensures the formation of plasmin, which is generated locally from plasminogen, and this digests the fibrin component of the thrombus leading to its partial or complete dissolution. Over the last quarter century there has been a great increase in the understanding of the molecular basis of this system, which in combination with the development of recombinant DNA technology, has lead to the enormous clinical benefit derived from the use of therapeutic thrombolytic agents. Thus, occlusive thrombus forms within a vessel when circumstances within the vessel allow the equilibrium between the pro-thrombotic and fibrinolytic systems to be disturbed in

favour of thrombosis. At the same time, by either using pharmacological thrombolytic agents, or possibly by enhancing endogenous fibrinolysis, it is possible to restore that equilibrium, and with it, vessel patency.

Plasminogen is a circulating proenzyme which is only converted to plasmin within the circulation in the presence of fibrin. Plasmin is able to digest fibrin and also fibrinogen, factor V and factor VIII. To ensure that fibrinolysis remains a local phenomenon, plasmin is rapidly inactivated by circulating alpha-2 antiplasmin, and to a lesser extent by alpha-2 macroglobulin and C1 esterase inhibitor.

The conversion of plasminogen to plasmin is brought about by plasminogen activators, of which 2 have been identified and these are presently available as commercially produced pharmacological agents. Named according to their initial site of identification, these are tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Although both are found within the circulation, it is t-PA which appears to be the more important generator of plasmin within the intravascular space, particularly as its activity is greatly enhanced in the presence of fibrin. Both t-PA and u-PA may exist as single chain (sc) or two chain (tc) molecules and t-PA is most active in its sc form.

The plasminogen activators are inhibited by 2 specific inhibitors, plasminogen activator inhibitor type 1 (PAI-1) and plasminogen activator inhibitor type 2 (PAI-2). PAI-1 is synthesised in the liver and by vascular endothelium, and is found in low free circulating concentrations but high concentrations within platelets (Booth et al 1988). PAI-2 was first identified in the placenta and can be detected in 2 molecular forms in the plasma of pregnant women, but is not normally detectable in

the plasma of healthy, non-pregnant individuals. Alpha-2 macroglobulin and C1 esterase inhibitor may also play a small role in inhibiting the plasminogen activators. Thus, the two most important components in the control of the fibrinolytic system appear to be t-PA and PAI-1. The overall activity of the endogenous fibrinolytic system is governed by a number of factors including:

(i) the presence of fibrin. Plasminogen and t-PA bind to fibrin polymer where they are favourably juxtaposed to generate plasmin in a site where it is to an extent protected from antiplasmin by the fibrin structure.

(ii) the endothelium. The endothelium is a site of production for both t-PA and PAI-1, and may respond to different stimuli for each of these. Local production of t-PA is likely to be of crucial importance in ensuring successful endogenous fibrinolysis (reviewed by Emeis in 1992). An important observation is that t-PA which is present prior to thrombus formation both impedes the formation of thrombus and accelerates the dissolution of thrombus by the addition of further t-PA in vitro, and in an animal model subthrombolytic doses of t-PA could prevent thrombus formation (Fox et al 1985). The endothelium may also play an important role in governing intravascular flow and other pro- or anti-thrombotic influences such as activating protein C, which binds and inactivates PAI-1, or prostacyclin, which inhibits further platelet aggregation.

(iii) the presence of inhibitors. Within the circulation, alpha-2 antiplasmin is the most important inhibitor and rapidly inactivates free plasmin. However, within an intravascular thrombus the concentrations of PAI-1 are much higher, probably because of the large platelet component, and in this setting PAI-1 may be the more

important. PAI-1 is found in the endothelial cells and smooth muscle cells of healthy human arteries but in diseased arteries there is increased expression of PAI-1 mRNA within the cellular component of the atheromatous plaque which may promote thrombus function at the site of plaque rupture (Lupu et al 1993).

(iv) The clearance of fibrinolytic components. Hepatic clearance of t-PA is rapid but the exact mechanism is unclear (Brommer et al 1988). PAI-1 is probably cleared as a complex with t-PA in the liver, but this is difficult to quantitate because the liver also produces PAI-1.

1.9 PHYSIOLOGICAL CONTROLS OF TISSUE-TYPE PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR TYPE I

The physiological pathways which control the activity of the endogenous fibrinolytic system are incompletely understood. Although a number of influences have been identified, it is difficult to place these in any hierarchical order.

It has long been recognised that there is a marked diurnal variation in endogenous fibrinolysis, with a peak activity in the afternoon and a nadir in the early morning hours (Fearnley et al 1957). It is of note that this mirrors the known circadian variation in the incidence of myocardial infarction (Muller et al 1986). This rhythm has now been shown to be mainly due to large variations in PAI-1, which peaks in the early morning, and much smaller variations in t-PA. This has been confirmed in normal subjects (Andreotti et al 1988; Grimaudo et al 1988) and in patients with stable (Angleton et al 1989) and unstable (Huber et al 1988) coronary

artery disease. The diurnal pattern does not appear to follow changes in insulin, cortisol or catecholamines (Chandler et al 1990).

Adopting upright posture is associated with an increase in overall fibrinolytic activity, a rise in t-PA antigen level, and no change in PAI-1 (Winther et al 1992). Physical exercise is associated with a rise in fibrinolytic activity (Hamouratidis et al 1988), and recently it has been shown that this is only partially accounted for by the exercise induced increase in circulating adrenaline (Chandler et al 1992). The role of adrenergic mechanisms in influencing fibrinolysis was investigated in Edinburgh by Cash, who showed that the rise in plasminogen activator caused by an intravenous infusion of adrenaline was unaffected by practolol and only partially antagonised by propranolol (Cash et al 1970). The possible effects of β adrenoceptor antagonism on fibrinolysis were recently reviewed (Teger-Nilsson 1991) but in a previous study of 20 patients with stable angina treated for 6 months with either metoprolol or epanolol (a β_1 selective antagonist with intrinsic sympathomimetic activity) I was unable to show any effect upon either t-PA or PAI-1 (Wright et al 1994). Other possible hormonal influences on haemostasis have been reviewed by Grant and Medcalf (Grant and Medcalf 1990) but I would like to concentrate on 2 which may be of clinical relevance - for one, angiotensin II, there is only preliminary evidence; and for the other, insulin, there is a large body of evidence. The most important issues here are which components of the fibrinolytic system are modulated by these hormones and to what extent they exert either an acute modifying action or a chronic influence on fibrinolytic balance, and whether this is direct or via secondary effects.

In a study of 10 volunteers, 4 normotensives and 6 with essential hypertension, Ridker et al investigated the effect of an intravenous infusion of angiotensin II upon t-PA and PAI-1 antigen concentrations (Ridker et al 1993a). Compared to only 4 controls who received a control infusion of dextrose, the infusion of angiotensin II was associated with a small fall in t-PA but caused a significant increase in PAI-1 concentrations, which occurred against the normal diurnal fall of PAI-1. This study was important because it suggested a potentially important relationship between the renin-angiotensin system and risk of thrombosis. The time course of the experiment suggests a direct effect of angiotensin II on PAI-1 but does not identify the site of release - most probably from liver but possibly from endothelium or platelets. It also suggested the possibility that ACE inhibitors might influence fibrinolysis and risk of thrombosis. Two in vitro studies have investigated this further; in one angiotensin I and angiotensin II were shown to stimulate expression of mRNA for t-PA and PAI-1 in cultured rat aortic smooth muscle cells and the effect of angiotensin I was abolished by the addition of captopril (van Leeuwen et al 1994), and in the other angiotensin I and angiotensin II stimulated PAI-1 mRNA production in cultured bovine aortic endothelial cells and the effect of angiotensin I could be abolished by captopril (Vaughan et al 1995). These studies support an effect of angiotensin II upon fibrinolysis within the vascular tree and also hint at the potential importance of the local renin-angiotensin system.

The evidence for a role of insulin in the physiological control of fibrinolysis was discussed earlier in this chapter and here I will discuss the possible influence of insulin on fibrinolysis in various pathological conditions. Vague and colleagues first

reported a significant positive correlation between fasting insulin and PAI-1 in normal and obese subjects (Vague et al 1986) and then went on to describe the same relationship in 2 groups of patients, 67 patients with angina pectoris, of whom only 15 had suffered previous myocardial infarction (Juhan-Vague et al 1989a), and 38 patients with non-insulin dependent diabetes mellitus (Juhan-Vague et al 1989b). Recently a study of 1484 patients with angina pectoris who were undergoing coronary angiography reported on the associations between fasting insulin concentrations and components of the fibrinolytic system (Juhan-Vague et al 1993a). The strongest relationships were between insulin and PAI-1 antigen and activity ($r = 0.44$, $p < 0.0001$) and between insulin and t-PA antigen ($r = 0.36$, $p < 0.0001$). The evidence for a link between hypofibrinolysis and insulin resistance was reviewed by Juhan-Vague who made two important points, firstly that the strength of the association between insulin and PAI-1 was very similar in all patient groups that had been studied, and secondly that the relationship was consistent only for fasting insulin concentrations, with less evidence for the importance of stimulated insulin responses (Juhan-Vague 1991). A positive correlation between PAI-1 and insulin resistance measured by the euglycaemic clamp has been shown in obese subjects (Landin et al 1990) and in non-insulin dependent diabetes (Potter van Loon et al 1993) but has not been studied in other patient groups.

The in vitro evidence for insulin increasing hepatic secretions of PAI-1 was reviewed earlier (Section 1.4). An alternative mechanism is that the hypertriglyceridaemia that is also a feature of the hyperinsulinaemic syndrome may be important, and this was supported by an experiment that showed that the very low

density lipoprotein (VLDL) particles from hypertriglyceridaemic subjects are a potent stimulus to PAI-1 secretions from endothelial cells in culture (Stiko-Rahm et al 1990). In a more recent study of hypertriglyceridaemic and normotriglyceridaemic men from the same investigators, they confirmed that hypertriglyceridaemic men had significantly higher PAI-1 activity, fasting insulin and stimulated hyperinsulinaemia (Asplund-Carlson et al 1993). In univariate analysis the usual relationship between fasting insulin and PAI-1 was present, but in multivariate analysis only VLDL triglyceride concentrations remained a significant predictor. However, the problems of performing multivariate analysis with variables that are so closely linked must be borne in mind.

Most information regarding the hyperinsulinaemic-hypofibrinolytic relationship has been gained from studies in subjects with non-insulin dependent diabetes mellitus and some interesting observations may be worthy of note. In a study of 51 fasting diabetics, Nagi et al found that a significant correlation was only present between PAI-1 and fasting levels of 32-33 split proinsulin, and not with total immunoreactive insulin (as measured in the standard assays) or total insulin (by specific IRMA assays) or proinsulin (Nagi et al 1990). However, the link between PAI-1 and both fasting immunoreactive insulin and a derived insulin resistance score has since been confirmed (Gough et al 1993).

Two studies have examined the effect on PAI-1 of chronic treatment of insulin resistant Type 2 diabetic patients with exogenous insulin, which might worsen the situation if hyperinsulinaemia is important or alternatively might improve it if overcoming insulin resistance is important. After 16 weeks of insulin treatment

in 19 patients with secondary failure to sulphonylureas there was no change in either t-PA or PAI-1 antigen concentrations, despite as improvement in glycaemic control (Vukovich et al 1992). However, in a crossover comparison of sulphonylureas or insulin in 11 Type 2 diabetics, there was a significant decrease in PAI-1 activity after 8 weeks treatment with insulin (Jain et al 1993), suggesting that overcoming insulin resistance might be important. Other therapeutic strategies may also have an influence. In a study in which insulin was not measured, the standard dietary advice issued to 14 newly diagnosed Type 2 diabetics was responsible for a significant fall in PAI-1 activity over 12 weeks (Bahru et al 1993), but there was no association between this and concomitant changes in glycaemic control, body mass index and serum triglycerides. Lastly, a number of studies in diabetic subjects have suggested that the biguanide oral hypoglycaemic agent metformin, which increases insulin sensitivity, improves fibrinolytic function in association with a suppression of PAI-1 (Grant 1991).

Thus, there is good evidence for basal insulin levels having a modulating influence on endogenous fibrinolysis via an effect on PAI-1, and possibly t-PA also. There is some evidence to suggest that this may, at least in part, reflect the disturbances of triglyceride metabolism associated with hyperinsulinaemia. There is no evidence to support an acute effect of exogenous insulin, and conflicting evidence of a chronic effect of exogenous insulin administration in non-insulin dependent diabetes mellitus.

1.10 CLINICAL STUDIES OF FIBRINOLYTIC PARAMETERS IN ISCHAEMIC HEART DISEASE

Disturbances of t-PA and PAI-1 have been described in many disease states (Kruithof et al 1988). Three recent reviews have specifically addressed their role in the pathogenesis of ischaemic heart disease (Hamsten 1993; Juhan-Vague and Alessi 1993b; de Bono 1994). The idea that fibrinolysis might be important in atherosclerotic disease is far from new (Astrup 1956) and early studies showed fibrinolytic function to be impaired both around the time of acute myocardial infarction (Ogston and Fullerton 1965) and in the convalescent phase after myocardial infarction (Chakrabarti et al 1968). These initial studies relied on tests of global fibrinolytic function, such as the euglobulin clot lysis time, and did not measure specific fibrinolytic components. Before reviewing more recent studies which have included assays of t-PA and PAI-1, two prospective studies are worthy of mention. Between 1972 and 1978, 1511 healthy white men aged between 40 and 64 years were recruited into the Northwick Park Heart Study. Initial results after a mean follow up of 10 years suggested that plasma fibrinogen and factor VII were the most important predictors of major ischaemic heart disease events (Meade et al 1986). However, after a mean follow up of 16 years, dilute blood clot lysis time was the most important predictor of a fatal or non-fatal events in men who were aged 40-54 years at enrolment (Meade et al 1993). In this study the haemostatic variables were consistently stronger predictors of events than plasma cholesterol. It only became apparent that fibrinolysis was important in the younger group after extended

follow up because it took that length of time for enough events to accrue in a group initially at lower risk. However, in the Goteborg study (Wilhelmsen et al 1984), which established fibrinogen as a risk factor for primary cardiovascular events, fibrinolytic activity, measured by euglobulin lysis on a fibrin plate, failed to distinguish those who had events during 13.5 years follow up. This population had a mean age of 54 years, but events were not specifically analysed with respect to age and therefore it is possible that impaired fibrinolysis is a risk factor for younger age groups only.

A large number of studies have now addressed the possible importance of t-PA and PAI-1 in patients with established ischaemic heart disease. An overview of these is represented in Tables 4, 5, and 6. Table 4 gives the results from studies of patients with stable ischaemic heart disease, the majority of whom were studied when they were attending for routine coronary angiography. A consistent observation from these studies was a lack of any relationship between the extent of coronary artery stenoses and fibrinolytic parameters. The principal finding was of elevation PAI-1 activity. PAI-1 antigen was usually increased when it had been measured, and interestingly, t-PA antigen levels also were higher than in controls. Measurements of t-PA activity in these groups did not reveal differences from controls. Three studies suggested that PAI-1 levels were higher in patients with a previous history of myocardial infarction than in those with stable angina (Aznar et al 1988; Juhan-Vague 1989a; The ECAT Angina Pectoris Study Group 1993).

Table 5 shows the results from studies of patients in the convalescent phase of myocardial infarction at times from 8 weeks to 3 years. The work of Anders

Hamsten was the most important in drawing attention to the possible relevance of PAI-1. He recruited male survivors of myocardial infarction who had been aged less than 45 years at the time of presentation and undertook a prospective cohort study. He initially identified that this group had elevated levels of the recently recognised PAI-1, accompanied by decreased t-PA activity in the presence of raised levels of t-PA antigen (Hamsten et al 1985). Subsequently he showed that elevated PAI-1 activity was a marker for increased risk of reinfarction, but did not report the relationship with baseline t-PA measurements (Hamsten et al 1987b). An interesting observation from other studies was that depressed t-PA activity and increased PAI-1 activity may be implicated in subjects who suffer myocardial infarction but have relatively trivial coronary artery disease (Verheugt et al 1987).

Table 6 displays data from studies in which t-PA and PAI-1 were measured around the time of presentation, and sometimes during follow up, in patients with the acute coronary syndromes of either unstable angina or acute myocardial infarction. Once again the most frequent finding was of elevated PAI-1 activity and in many studies t-PA antigen concentrations were also increased. Several studies suggested that PAI-1 activity was higher in those with acute myocardial infarction, and therefore with presumed occlusive thrombosis, than in those with unstable angina. A very interesting suggestion, first proposed by Lucore, was that in patients treated with t-PA thrombolytic therapy, there may be a rebound increase in PAI-1 at the end of the infusion which might be associated with an increased risk of failure to achieve vessel patency or for reocclusion to occur (Lucore et al 1988). Evidence has been provided from a number of angiographic studies to support this (Barbash et al 1989;

Andreotti et al 1990; Sane et al 1991) and in a prospective study using non-invasive markers to assess reperfusion (Gray et al 1993c).

The most damaging criticism of much of this work, however, is that the case-control design of most of these studies does not answer the question of whether the disorders of fibrinolytic parameters encountered in patients with established disease represents cause or effect. The important early studies which outlined that these factors might indicate risk of *subsequent* events were those of Hamsten showing that greater PAI-1 activity predicted risk of reinfarction (Hamsten et al 1987b), and of Gram, which received much less attention and which suggested that decreased t-PA activity and, counter-intuitively, increased t-PA antigen also predicted risk of reinfarction (Gram et al 1987a and 1987b). Later, two studies found that in patients with unstable or severe stable angina pectoris baseline levels of t-PA antigen, and not PAI-1, predicted the likelihood of future myocardial infarction (Munkvad et al 1990a; Jansson et al 1991). These studies helped to underline that disturbances of endogenous fibrinolysis might play a causal role in the future development of myocardial infarction, but two pieces of stronger evidence were to be published in 1993.

In the US Physicians' Health Study, designed to investigate the place of aspirin in primary prevention, baseline blood samples were taken and plasma stored. After a mean of 5 years, 231 individuals had developed myocardial infarction and they were matched for controls from within the same study who remained free of disease (Ridker et al 1993b). Plasma concentrations of t-PA antigen, but not of PAI-1, were significant predictors of the risk of infarction. This suggested that disorders

of endogenous fibrinolytic precede the development of acute syndromes associated with occlusive thrombosis by many years. Further support for this hypothesis came from the same study when Ridker reported that the risk of stroke was similarly predicted by baseline levels of t-PA antigen (Ridker et al 1994). A recent study has supported a role for altered fibrinolysis in the early stages of atherosclerosis in asymptomatic individuals in whom those with increased intima-media thickness of the carotid artery had elevated plasma concentrations of tPA and PAI-1 antigen (Salomaa et al 1995). Furthermore, in a follow up of their study first published in 1991, Jansson et al reported that in 213 patients who had presented with severe angina pectoris, baseline concentrations of t-PA antigen, and not PAI-1, predicted mortality at a mean follow up of 7 years (Jansson et al 1993). Of special note, this finding also applied to the 78% of patients in this group who had undergone coronary artery bypass grafting shortly after their initial presentation. These findings have now been confirmed in a large cohort study of 3043 patients with chronic stable angina followed up for 2 years in whom an increased risk of myocardial infarction or sudden death was associated with higher baseline concentrations of t-PA antigen (Thompson et al 1995).

Thus, although initial impressions were that it was measurements of PAI-1 activity which were indicative of the importance of endogenous fibrinolysis, the pendulum has now swung in favour of the assay of t-PA antigen. This apparent paradox is perhaps not too difficult to explain. Firstly, t-PA and PAI-1 can either be measured in terms of their total mass concentration by measuring their antigenicity in plasma, or in terms of their functional ability. The majority of t-PA and PAI-1 in the

circulation exists in a 1:1 complex which is functionally inert but has maintained antigenicity. Short term variations in fibrinolytic activity seem to be governed by changes in the release of PAI-1, and there is good evidence that thrombosis induces both the secretion of PAI-1 (Fujii et al 1991) and the expression of PAI-1 mRNA (Fujii et al 1992). The majority of t-PA that is secreted becomes quickly bound to PAI-1 and therefore exists in the functionally inactive complexed form, whilst the minority exists in a free, functionally active form. This explains the positive correlation that is reported between PAI-1 antigen and activity, and between PAI-1 activity and t-PA antigen, but the negative correlation between t-PA antigen and activity (Olofsson et al 1989; Chandler et al 1994). Perhaps because of the marked diurnal variation in PAI-1, it appears that plasma concentrations of t-PA antigen more accurately reflect the overall activity of the fibrinolytic system and, by implication, the overall pro-thrombotic tendency. The recent evidence suggests that both of these may be abnormally elevated for many years prior to a failure of the fibrinolytic system leading to a clinical thrombotic event.

1.11 SUMMARY

This review has attempted to present the evidence for hyperinsulinaemia and endogenous fibrinolysis being implicated in the pathogenesis of ischaemic heart disease. In addition, the possible links between the two have been discussed. The most important points are:

- (1) Insulin has many diverse actions mediated through more than one type of receptor and these include effects upon metabolism, the autonomic nervous system, vascular tone, electrolyte balance, cell growth and arterial structure, thrombosis and fibrinolysis.
- (2) There is evidence to support an aetiological role for hyperinsulinaemia in the development of myocardial infarction and hyperinsulinaemia is a feature of patients with established ischaemic heart disease.
- (3) Hyperinsulinaemia usually reflects underlying resistance to the effects of insulin on glucose metabolism. To what extent insulin resistance extends to other actions of insulin is unclear.
- (4) It is possible to modify hyperinsulinaemia in patients with hypertension and diabetes mellitus using drugs and/or non-pharmaceutical intervention.
- (5) Occlusive vascular thrombosis occurs when there is an imbalance between the pro-thrombotic forces and the endogenous fibrinolytic system.
- (6) Disturbances of tissue plasminogen activator and plasminogen activator inhibitor type 1 are a feature of patients with ischaemic heart disease and may be used to predict prognosis.

- (7) Insulin appears to be an important modulator of endogenous fibrinolysis. An association between hyperinsulinaemia and impaired endogenous fibrinolysis has been shown in subjects with obesity, non-insulin dependent diabetes mellitus, and ischaemic heart disease.

Rationale for the work performed for this MD Thesis

I have attempted to extend previous observations relating to the nature of hyperinsulinaemia in ischaemic heart disease and to further investigate the relationship between insulin resistance and endogenous fibrinolysis in patients with ischaemic heart disease. When seeking to discover whether it was possible to modify either or both of these I was influenced by the recent studies suggesting that angiotensin converting enzyme inhibitors improve prognosis in patients with mild left ventricular dysfunction following myocardial infarction (Pfeffer et al 1992; The SOLVD Investigators 1991 and 1992) and by the unexpected reduction in the incidence of acute coronary syndromes in these studies (Pfeffer et al 1992; Yusuf et al 1992). I have sought to answer the following questions:

- (1) Is the extent of hyperinsulinaemia in patients with ischaemic heart disease influenced by the extent of left ventricular impairment or by a history of myocardial infarction as opposed to chronic stable angina.
- (2) Impaired glucose tolerance is a risk factor for developing ischaemic heart disease and is associated with hyperinsulinaemia and insulin resistance. The incidence of impaired glucose tolerance is high in patients with ischaemic

heart disease and I sought to answer whether this further increased degree of hyperinsulinaemia.

- (3) Insulin resistance in patients with ischaemic heart disease has not been quantified using the hyperinsulinaemic euglycaemic clamp. Insulin resistance may be favourably modified by angiotensin converting enzyme inhibitors in some patient groups. I have quantified insulin resistance in patients with previous uncomplicated myocardial infarction and investigated whether it is modified by the angiotensin converting enzyme inhibitor captopril.
- (4) In this same patient group I have investigated the relationship between hyperinsulinaemia, insulin resistance, and components of the endogenous fibrinolytic system. The effect of captopril on tissue-type plasminogen activator and plasminogen activator inhibitor type 1 was defined.

TABLE 1. EVIDENCE FOR FASTING OR STIMULATED HYPERINSULINAEMIA IN PATIENTS WITH ISCHAEMIC HEART DISEASE UNDERGOING AN ORAL GLUCOSE TOLERANCE TEST

Study	Sample size (patient/ control)	MI patients only	Exclusion Criteria			Results		
			Obesity	HT	IGT	DM	Fasting hyperinsulinaemia	Stimulated hyperinsulinaemia
Peters et al, 1965	7/7	Y (< 17/12)	N	Y	Y	N	Y	Y
Nikkila et al, 1965	29/18	Y (< 5/52)	N (33%)	N (29%)	Y	N	N	Y
Tzagournis et al, 1967	25/21	N	Y	N (40%)	Y	N	N	Y
Tzagournis et al, 1968	50/30	N	N	N (34%)	Y	N	N	Y
Kashyap et al, 1970	18/12	N	Y	N	N	Y	N	Y
Malherbe et al, 1971	20/20	Y (< 10 yrs)	Y	N (25%)		N	N	Y
Gertler et al, 1972	65/69	N	N	N (37%)	Y	N	N	N (Y in IGT group)
Berchtold et al, 1972	43/81	Y (< 3/12)	N	N	N	N	N	Y
Sorge et al, 1976	127/89	Y	N (50%)	N (50%)	Y	N	N	Y
Bergstrand et al, 1979	20/40	Y (< 6 yrs)	N	N (33%)	Y	N	N	N
Larsen et al, 1981	10/10	Y	N	Y	Y	N	Y	Y
Hamsten et al, 1987a	104/100	Y	Y	N (33%)	Y	N	N	Y
Jackson et al, 1983	7/8	Y	Y	Y	Y	N	N	N
Lichtenstein et al, 1987	499/1526	N	N	N	N	N	Y	ND
Young et al, 1993	20/20	N	Y	N	Y	Y	N	Y
Chauhan et al, 1994	17/17	N	Y	N	Y	Y	N	Y

MI = myocardial infarction, IGT = impaired glucose tolerance, DM = diabetes mellitus, HT = hypertension, ND = not done

TABLE 2. DEFECTS IN INSULIN-MEDIATED GLUCOSE METABOLISM ASSESSED BY THE EUGLYCAEMIC CLAMP (ADAPTED FROM DE FRONZO AND FERRANNINI 1991)

	Obesity	NIDDM	Hypertension
Whole body glucose uptake	↓↓↓	↓↓↓	↓↓
Glucose oxidation	↓	↓	0
Non-oxidative glucose disposal (mainly glycogen synthesis)	↓↓	↓↓	↓↓
Suppression of hepatic glucose production	↓	↓	0

NIDDM = non-insulin dependent diabetes mellitus

TABLE 3. EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS ON HYPERINSULINAEMIA AND INSULIN RESISTANCE

Study	Drug	Subjects	n	Design	Duration	Placebo control	Glucose tolerance	Fasting insulin	Stimulated insulin	Insulin sensitivity
Pollare, 1989b	captopril 50 mg bd	HT	24	parallel group	4/12	Y	→	→	↓ (ivgtt)	↑ (clamp)
Seefeldt, 1990	enalapril 20 mg daily	IDDM	8		3/52	N	→ (HbA _{1c})	→	ND	→ (clamp)
Torlone, 1991	captopril 50 mg bd	NIDDM with HT	12	crossover single-blind	2/7	Y	ND	→	ND	↑ (clamp)
Gans, 1991	enalapril 10 mg bd	Normals	7	sequential	1/52	N	ND	→	ND	↑ (clamp)
Alleman, 1992	fosinopril 20 mg od	Normals	24	parallel group	3/52	Y	→	→	→	→ (fsivgtt)
Santoro, 1992	cilazapril 5 mg daily	HT	20	sequential	1/12 placebo 3/12 cilazapril	Y	↓	→	→	→ (clamp)
Seghieri, 1992	captopril	NIDDM with HT	14	sequential	3/12	N	→	→	→	→ (IST)
Bak, 1992	perindopril 4 mg od	NIDDM with HT	10	crossover double-blind	6/52	Y	→	→	→	→ (ivgtt)
Egan, 1993	enalapril 10 mg bd	HT with obesity	9	sequential low Na diet	2/52	N	→	→	↓	ND
Baba, 1993	enalapril 10 mg od	HT	8	sequential	6/12	N	→	→	→	→ (fsivgtt)
Torlone, 1993	captopril 25 or 50 mg bd	NIDDM with HT	16	crossover single-blind	3/12	Y	↓ (HbA _{1c})	→	ND	↑ (clamp)
Uehara, 1994	captopril (C) 25 mg	Normals HT	8	sequential	single dose	N	ND	ND	ND	↑ (C)
	enalapril (E) 20 mg	NIDDM	8				ND	ND	ND	→ (E)
	delapril (D) 75 mg	NIDDM with HT	8				ND	ND	ND	→ (D)
Okasa, 1994	enalapril 5-40 mg	HT	19	sequential	8-12/52	N	↓	→	→	ND
	lisinopril 20-40 mg		19						(↓ in IGT group)	
	perindopril 4-8 mg		14							

HT = hypertension, IDDM = insulin dependent diabetes mellitus, NIDDM = non-insulin dependent diabetes mellitus, IGT = impaired glucose tolerance, ND = not done, IVGTT = intravenous glucose tolerance test, FSIVGTT = frequently sampled IVGTT, IST = insulin suppression test, ↑ = increased, → = no change, ↓ = decreased

TABLE 4. STUDIES OF ENDOGENOUS FIBRINOLYSIS IN STABLE ISCHAEMIC HEART DISEASE

Study	n	t-PA Ag	t-PA Act	PAI-1 Ag	PAI-1 Act	Other	Comments
Paramo, 1985	n=118	↑			↑	→ ECLT	No relationship with the extent of coronary disease. 26 on β blockers - no δ.
Mehta, 1987	n=60		?		↑		PAI-1 correlated with TG. No data on t-PA given.
Aznar, 1988	SA n=15 SA+old MI n=14 3/52 post MI n=38	↑			↑		Those with a previous MI had ↑ t-PA Ag, ↑ PAI-1 Act and ↓ t-PA Act versus SA only.
Francis, 1988	99				↑		↓ Release of t-PA Ag and t-PA Act after venous occlusion. Basal levels not given.
Vandekerckhove, 1988	Coronary occlusion n=29 Coronary disease n=18 No coronary disease n=24	→	→				No difference in t-PA levels, but the control group included diabetics and hypertensives.
Juhan-Vague, 1989a	SA n=22 VA n=17 UA n=24 MI n=15	↑		↑	↑	MI v rest MI v rest	

t-PA Ag = tissue-type plasminogen activator antigen, t-PA Act = tissue-type plasminogen activator activity, PAI-1 Ag = plasminogen activator inhibitor 1 antigen, PAI-1 Act = plasminogen activator inhibitor 1 activity, ECLT = euglobulin clot lysis time, SA = stable angina, VA = variant angina, UA = unstable angina, MI = myocardial infarction, TG = plasma triglyceride, ↑ = increased, → = no change, ↓ = decreased

TABLE 4 (continued). STUDIES OF ENDOGENOUS FIBRINOLYSIS IN STABLE ISCHAEMIC HEART DISEASE

Study	n	t-PA Ag	t-PA Act	PAI-1 Ag	PAI-1 Act	Other	Comments
Olofsson, 1989	Severe angina n=214	↑			↑		The highest levels were in smokers and hypertensives.
Oseroff, 1989	Angina (no MI) n=65		→		→		No correlation with the extent of coronary disease. The control group were chest pain/normal coronary arteries i.e. not normals.
Huber, 1990	CAD n=16 NCA n= 15	↑			↑		No correlation with extent of coronary disease. Tend to be higher in those with old MI.
Rydzewski, 1990	CAD n=45 Normals n=23	↑ ns		↑ ns	↑ ns		
Sakata, 1990	CAD n=47	→	→	→	↑		↑ PAI-1 Act in multi-vessel disease.
ECAT, 1993	CAD n=2317 NCA n=726	↑	→	↑	↑	↑ ECLT	CAD v NCA: ↑ fibrinogen, plasminogen. MI v no MI: ↑ PAI-1, → t-PA. Smokers: ↑ fibrinogen, PAI-1, t-PA Ag.
Jansson, 1993	Severe angina n=213 46% had old MI	↑					↑ t-PA Ag was the only independent predictor of mortality at 4 and 7 year follow up. PAI-1 activity did not predict mortality (see also Jansson, 1991).

t-PA Ag = tissue-type plasminogen activator antigen, t-PA Act = tissue-type plasminogen activator activity, PAI-1 Ag = plasminogen activator inhibitor 1 antigen, PAI-1 Act = plasminogen activator inhibitor 1 activity, CAD = coronary artery disease, NCA = normal coronary arteries, MI = myocardial infarction, ECLT = euglobulin clot lysis time. ns = not significant, ↑ = increased, → = no change, ↓ = decreased

TABLE 5. STUDIES OF ENDOGENOUS FIBRINOLYSIS IN THE RECOVERY PHASE OF ACUTE MYOCARDIAL INFARCTION

Study	Time from MI	n	t-PA Ag	t-PA Act	PAI-1 Ag	PAI-1 Act	Other	Comments
Hamsten, 1985	3 years (age < 45)	71	↑	↓		↑		
Hamsten, 1986	3-6/12 (age < 45)	148		↓		↑	↓ ECLT	No correlation with extent of coronary disease.
Hamsten, 1987b	3-6/12	109				↑	↓ ECLT	↓ t-PA Act and ↑ PAI-1 Act in those with reinfarction (16/109).
Gram, 1987a	8/52	29	↑					↓ t-PA Act in 9/29 with reinfarction over 4 years.
Gram, 1987b	8/52	29					↓ ECLT	↑ t-PA Ag in 9/29 with reinfarction over 4 years
Nilsson, 1987	12/52	124	↑ (after VO only)	↓		↑		
Verheught, 1987	3 years	MI+NCA n=18 MI+CAD n=18						MI+NCA had ↓ t-PA Act v MI+CAD and v normals. MI+NCA had ↑ PAI-1 Act v normals.
Wiman & Hamsten, 1990	3 years	133 (age < 45)	↑	↓		↑	↓ ECLT	
Gray, 1993b	6-24/12	MI n=50 MI+NIDDM n=24	↑		↑	↑		The disturbances in fibrinolysis were similar in those with and without NIDDM.

t-PA Ag = tissue-type plasminogen activator antigen, t-PA Act = tissue-type plasminogen activator activity, PAI-1 Ag = plasminogen activator inhibitor 1 antigen, PAI-1 Act = plasminogen activator inhibitor 1 activity, ECLT = euglobulin clot lysis time, CAD = coronary artery disease, NCA = normal coronary arteries, MI = myocardial infarction, NIDDM = non-insulin dependent diabetes mellitus, VO = venous occlusion, ↑ = increased, → = no change, ↓ = decreased

TABLE 6. STUDIES OF ENDOGENOUS FIBRINOLYSIS IN ACUTE CORONARY SYNDROMES

Study	n	t-PA Ag	t-PA Act	PAI-1 Ag	PAI-1 Act	Timing of samples	Comments
Almer, 1987	AMI n=29				↑	0 and 1/52	↑ PAI-1 Act stable over 1/52.
Huber, 1988	AMI n=36 UA n=27	→			↑	6 hrly for 48 hrs	PAI-1 higher in MI v UA. Diurnal variation was preserved.
Lucore, 1988	AMI n=21	→			↑	hrly for 9 hrs, daily for 1/52	t-PA Ag was stable day 2-7. 50% of cases showed a rebound ↑ in PAI 1 Act after cessation of rt-PA.
Barbash, 1989	AMI n=125 (all had rt-PA)	↑			↑	0, 4, 24, 48 hrs	↑ t-PA Ag and ↑ PAI-1 Act at baseline in those whose artery was occluded at 4 /7.
Andreotti, 1990	AMI n=24 (all had rt-PA)				↑	0, 24, 48, 72 hrs and 90 days	PAI-1 Act peak at 24 hrs, but 0, 2 and 90 day values were similar. Patients with occluded arteries had higher PAI-1 Act.
Munkvad, 1990a	UA n=20	↑	↓	↑ (ns)	↑ (ns)	24 hrs	Over a 6 year follow up, 8/20 → MI. These had ↑ t-PA Ag, ↓ t-PA Act, and tended to have ↑ PAI-1 Ag and Act.
Munkvad, 1990b	UA, n=20 v AMI, n=34	→	↓	→	↑	24 hrs	Note these comparisons are AMI v UA.

t-PA Ag = tissue-type plasminogen activator antigen, t-PA Act = tissue-type plasminogen activator activity, PAI-1 Ag = plasminogen activator inhibitor 1 antigen, PAI-1 Act = plasminogen activator inhibitor 1 activity, AMI = acute myocardial infarction, UA = unstable angina, rt-PA = recombinant tissue-type plasminogen activator, ns = not significant, ↑ = increased. → = no change, ↓ = decreased

TABLE 6 (continued). STUDIES OF ENDOGENOUS FIBRINOLYSIS IN ACUTE CORONARY SYNDROMES

Study	n	t-PA Ag	t-PA Act	PAI-1 Ag	PAI-1 Act	Timing of samples	Comments
Alexopoulos, 1991	UA, n=26 v SA, n=25				→	48 hrs	No difference in PAI-1 Act between stable and unstable angina, but may have sampled too late. Did not account for diurnal rhythm.
Rapold, 1991	AMI n=30 (all had rt-PA)			↑	↑	0, 1.5, 2, 3.5, 12, 24 hrs	↑ PAI-1 Ag and Act at baseline and these peak at 12 hrs.
Sane, 1991	AMI n=386 (all had rt-PA)			↑	↑	0, 6, 12 hrs	↑ PAI-1 Ag in those with occluded coronary artery.
Zalewski, 1991	UA, n=17 v SA, n=10	→	→	→	↑	0 hrs	PAI-1 Ag and Act did not predict reinfarction.
Munkvad, 1992	AMI n=20 (all had rt-PA)	↑		↑	↑	0, 2, 4, 12, 24, 72, 96 hrs	Only PAI-1 differentiated UA and SA. In UA, fibrinolytic activity tended to improve prior to discharge. t-PA Ag peaked at 2 hrs. PAI-1 peaked at 12 hrs and was stable by 72-96 hrs.
Sakamoto, 1992	AMI n=47	↑			↑	0 and 4 weeks	PAI-1 tended to be higher in those with occluded arteries. t-PA Ag and PAI-1 were ↑ on admission and fell by 4/52, but were still higher than controls.
Sakata, 1992	VA, n=15 SA, n=9 v NCA, n=19	↑		↑		6 hrly for 24 hrs	↑ PAI-1 Ag in VA v UA. Diurnal variation preserved.
Gray, 1993a	AMI, n=90 AMI+NIDDM, n=38			↑	↑	0 hrs	↑ PAI-1 in all patients with AMI. ↑ PAI-1 in NIDDM v non-NIDDM.

t-PA Ag = tissue-type plasminogen activator antigen, t-PA act = tissue-type plasminogen activator activity, PAI-1 Ag = plasminogen activator inhibitor 1 antigen, PAI-1 Act = plasminogen activator inhibitor 1 activity, SA = stable angina, VA= variant angina, UA = unstable angina, AMI = acute myocardial infarction, rt-PA = recombinant tissue-type plasminogen activator, NCA = normal coronary arteries, NIDDM = non-insulin dependent diabetes mellitus, ↑ = increased, → = no change, ↓ = decreased

CHAPTER 2

A REVIEW OF METHODS USED IN THIS THESIS

2.1 INTRODUCTION

In this chapter I shall review briefly some aspects of the methods which have been employed in the studies described in this thesis. Where relevant I shall describe any validation work that I have performed.

2.2 ORAL GLUCOSE TOLERANCE TEST

The cornerstone of the data presented in Chapters 3 and 4 is the 75 g oral glucose tolerance test (OGTT) as described by the World Health Organisation (World Health Organisation 1985). Important factors in the performance of this test include asking the subjects to (i) fast for 10-12 hours; (ii) take an unrestricted diet including at least 150 g of carbohydrate for 3 days prior to the test; and (iii) not smoking on the day of the test and avoiding alcohol consumption for 24 hours prior to the test. I routinely performed the tests with subjects seated and I placed an intravenous cannula for blood sampling 30 minutes prior to commencing the test. The cannula was flushed between samples with normal saline or "Hepsal," a solution of 10 U/mL heparin. Prior to sampling, 5 mL of blood was withdrawn and discarded to exclude the "deadspace."

The standard 75 g of glucose monohydrate in 200 mL water was administered over less than 3 minutes. Further blood samples were taken at 30 minute intervals for at least 2 hours. Samples for measurement of plasma glucose were taken into fluoride oxalate anticoagulant to prevent glucose uptake into red cells, and samples for

measurement of plasma insulin were taken into lithium heparin, placed on melting ice and plasma separated within one hour by centrifugation and stored at -40°C . Plasma glucose was measured by the hexokinase method (Schmidt 1973) on a Cobas Bio analyser. Plasma insulin was measured by radioimmunoassay using the double antibody technique (Soeldner and Slone 1965) by kind arrangement with Professor George Alberti at the Department of Medicine at the University of Newcastle Upon Tyne. Important aspects of the insulin assay are discussed later.

The OGTT has many advantages in clinical research. It is easily understood by the subject and is widely acceptable by being minimally invasive. It provides the simplest, although crude, estimation of insulinogenic indices in terms of the fasting insulin concentration, the 2 hour insulin concentration or the area under the insulin curve. In addition all of these may be corrected for the prevailing glucose concentration. It is probably best to use the OGTT to quantify fasting and stimulated insulin responses and not to infer from these tissue insulin sensitivity because that is inevitably derived from the former. The validity of using the OGTT in non-diabetic subjects to infer estimates of insulin secretion and insulin resistance was recently confirmed for studies comparing different patient populations (Phillips et al 1994). However, it must be remembered that under circumstances where insulin secretion is altered (e.g. non-insulin dependent diabetes mellitus or by certain drugs) then it is difficult to interpret, and it also fails to give direct information on tissue sensitivity to insulin. It is interesting to note that it is only in the last 10 years that it has become firmly established that the majority of glucose disposal ($\sim 75\%$) after an oral glucose

load is into insulin-sensitive peripheral muscle and that this is associated with an ~50% reduction in hepatic glucose output (Ferrannini et al 1985).

2.3 INSULIN ASSAYS

The principle of the radioimmunoassay used in Newcastle is the competition between a constant amount of ^{131}I -labelled insulin and the sample insulin for the available binding sites on a fixed amount of guinea-pig anti-insulin serum (Soeldner and Slone 1965). Separation of soluble antibody-bound insulin is facilitated by using an antibody raised in rabbits to guinea-pig globulin (the double antibody technique). Important features of this assay are its coefficient of variation which is 6.8-7.5%, and that it shows less than 20% cross reactivity with proinsulin and 32,33-split proinsulin. There has been considerable concern recently that many radioimmunoassays may significantly overestimate plasma insulin concentrations because of a combination of high cross-reactivity of the assay with abnormally high circulating levels of proinsulin and its split products (see Temple et al 1992, for a review). However, it now seems that it may only be in non-insulin dependent diabetics who exhibit poor glycaemic control that concentrations of these components are high (Temple et al 1990) and obscure a real insulin deficiency (Temple et al 1989). In diet-controlled diabetics (Clark et al 1992), in subjects with impaired glucose tolerance (Davies et al 1993), and in subjects with normal glucose tolerance who are at high risk of developing non-insulin dependent diabetes (Haffner et al 1994), these molecules comprise < 20% of the total immunoreactive insulin

measured by radioimmunoassay. Because the insulin assay used for my studies has < 20% cross reactivity with proinsulin and its split products, and because only non-diabetic subjects have been included in my studies, that implies that < 5% of the total immunoreactive insulin measured in my studies could be accounted for by proinsulin or its split products.

2.4 ASSESSMENT OF INSULIN SENSITIVITY

A number of approaches to quantifying insulin sensitivity have been developed by many different investigators but it is interesting to note that the conclusions of a review of these methods written over a decade ago (Reaven 1983) and of several more recent ones (Starke 1992; Groop et al 1993; Donnelly and Morris 1994) are not very different; the majority of investigators favour the euglycaemic hyperinsulinaemic clamp (De Fronzo et al 1979). Briefly, the main requirement of any of these techniques is that they reveal a measurable relationship between the plasma concentration of insulin and some quantifiable action of insulin on glucose metabolism. The tests employed should ideally fulfil a number of additional specifications which include (i) that the plasma insulin concentration is sufficient to suppress hepatic glucose production, so that the rate of glucose uptake is not underestimated; (ii) that a steady state condition is produced during which measurements can be made; (iii) that the plasma glucose concentration during the measurement of insulin sensitivity does not itself influence the result because,

particularly at low insulin concentrations, glucose uptake is partly dependent upon plasma glucose concentration.

The two most commonly used alternative methods to the clamp are the frequently sampled intravenous glucose tolerance test (FSIVGTT) in combination with a mathematical model, and the insulin suppression test (IST) using a combined infusion of insulin and glucose with or without the addition of somatostatin or adrenaline and propranolol. Each of these has their own merits, and in particular both are less laborious than the clamp, but there are some drawbacks. The mathematical model usually used with the FSIVGTT (Bergman 1989) inevitably makes assumptions about the compartments in which events take place, such that insulin action is in a “remote” compartment, but it does provide detailed information on the early and late phases of insulin secretion. The IST involves a continuous constant rate infusion of glucose and insulin, with either somatostatin (Harano et al 1977) or adrenaline and propranolol (Greenfield et al 1981) to suppress insulin secretion, and the steady state plasma glucose which is achieved after 2-3 hours is used as a measure of insulin sensitivity. The disadvantages of this technique are that the steady state plasma glucose concentration is often high and thus might influence its own uptake, that a true steady state is difficult to achieve, and that both intravenous infusion of somatostatin and of adrenaline are not without hazard or side effects.

2.4.1 THE EUGLYCAEMIC HYPERINSULINAEMIC CLAMP

For the studies reported in Chapter 5 of this thesis I used this technique as originally described in 1979 by De Fronzo. The essence of this method is that the

plasma insulin concentration is raised to a level at the upper end of the physiological range and the plasma glucose concentration is “clamped” at the basal level by a variable rate glucose infusion. The rate of the glucose infusion is adjusted according to frequent blood glucose determinations and when a steady state has been achieved, the glucose infusion rate is equal to the rate of whole body glucose disposal (M value, expressed in mg/kg.min). The M value should be corrected for the prevailing plasma insulin concentration to provide the most accurate measure of insulin sensitivity.

Clamp protocol

Subjects attended the department at 08.30 hours having observed the same restrictions as for the oral glucose tolerance test. They lay recumbent on a couch for the whole procedure and were asked to place their left hand within a heated water jacket in the shape of an open-ended cylinder which allows the hand to be entered to beyond the wrist and a thermometer to enter from the opposite end before the ends of the cylinder were sealed with foam and surgical dressing. The temperature inside the arm heater was kept between 55 and 65°C by altering the thermostatic control on the water heater. The purpose of heating the hand is to “arterialise” the blood in the veins of the dorsum of the hand. Recently, possible problems with this technique have been raised (Anstrup et al 1988; Gallen and MacDonald 1990) depending on the method of heating and I therefore undertook a validation of my method which is presented in the next section.

After 10 minutes a 21 G intravenous cannula was inserted retrogradely into a vein on the dorsum of the left hand and connected to a 3-way tap and continuous

(2 mL/hr) saline flush system. An 18 G cannula was inserted into a large antecubital vein and connected via a 3-way tap to both the 20% glucose solution (with 20 mmol KCL added to 500 mL 20% glucose), which was delivered via an IMED Gemini infusion pump, and the insulin infusion, given via a Graseby 50 mL syringe driver. The insulin solution was made of 20 units Human Actrapid insulin (Novo) in 45 mL of normal saline with 5 mL of the subjects own blood added to prevent absorption of the insulin onto plastic surfaces. The subjects rested for 30 minutes and 2 arterialised venous blood samples were taken 15 minutes apart to confirm that the blood glucose level was stable (< 0.2 mmol difference) prior to commencing the study. The mean of these two results was used as the target blood glucose concentration for clamp studies in that subject.

The insulin infusion was given at a constant rate of 40 mU/m^2 body surface area min after a 10 minute priming infusion given according to the schedule in Table 7. The aim of this regime is to raise the plasma insulin concentration to $\sim 100 \text{ mU/L}$ during the period of the clamp.

The glucose infusion was commenced after 4 minutes at a rate of 2 mg/kg.min , and increased at 10 minutes to 2.5 mg/kg.min . Thereafter the rate is adjusted according to the formula described by De Fronzo which is dependent upon measurements of blood glucose made at 5 minute intervals.

$$S_i = SV_i + SM_i$$

where for time i:-

S_i = the glucose infusion rate (mL/hr) at time i

SV_i = that portion of the glucose needed for the volume component

SM_i = that portion of the glucose needed for the metabolic component

$$SV_i = (G_d - G_i) \times (0.684 \times \text{body weight})$$

where:-

G_d = desired blood glucose concentration

G_i = blood glucose concentration at time i

$$SM_i = SM_{i-2} \times (G_d/G_i) \times (G_d/G_{i-1})$$

where:-

SM_{i-2} = SM_i from 10 minutes earlier

G_{i-1} = G_i from 5 minutes earlier

These calculations can be performed very quickly on a pocket calculator, despite their appearance, and all calculations were recorded on a custom form for each individual. In practice, and after personal discussion with Professor Alberti and Dr Ferrannini, the investigator may override the result of these equations in order to achieve a steady state more quickly once he becomes familiar with the technique, but the results from the calculations provide a good estimate of the correct infusion rate.

The target for the investigator was to maintain the blood glucose level at G_d (or within 0.1 mmol/L) for at least the last 60 minutes of the clamp. The blood glucose level was measured every 5 minutes using an autoanalyser based on the glucose oxidase method (Yellow Springs Institute, Yellow Springs, Ohio). This provides a result within 90 seconds of the sample being presented.

Samples for blood glucose were taken into 2 mL tubes containing fluoride oxalate anticoagulant. Samples for plasma insulin determination were taken into 5mL lithium heparin tubes and placed onto melting ice and treated as described for the oral glucose tolerance test. Samples for insulin assay were taken at -15, 0, 60, 70, 80, 90, 100, 110, and 120 minutes, in order to know the prevailing plasma insulin concentration during the last hour of the clamp.

At the end of the study, the insulin infusion was switched off and the 20% glucose infusion was continued for 30 minutes to prevent rebound hypoglycaemia which occurs as a result of the inhibition of hepatic glucose production. A measurement of plasma potassium was taken at the end of the study to ensure that adequate potassium replacement had been given over the course of the clamp, which proved to be so in every case.

The measure of insulin sensitivity used for my studies was the quotient of the whole body glucose disposal over the last hour and the prevailing plasma insulin concentration over the last hour.

2.4.2 “ARTERIALISED” VENOUS BLOOD SAMPLING

For all of the methods of measuring insulin sensitivity it has been emphasised that arterial blood glucose monitoring is to be preferred because, under the conditions of these methods, the circulating insulin concentration is elevated and the extraction of glucose across the capillary bed will be increased. However, the insertion of intra-arterial cannulae is regarded as unacceptable for routine studies by most investigators and instead they opt to heat the hand to open up arterio-venous channels and make

the venous blood “arterialised.” A cannula inserted retrogradely in the dorsum of the hand then samples blood which has undergone very little extraction of oxygen or nutrient molecules. Recently the use of warmed electric blankets to heat the arm has been criticised (Anstrup et al 1988) and other techniques to heat the air around the hand in a closed box have been developed (Gallen and MacDonald 1990). I opted to design my own system, partly for reasons of economy. With the help of Dr Riemersma, we constructed a cylindrical water jacket inside which the hand could rest to beyond the wrist and was supported so as to avoid direct contact with the wall. A thermometer was placed next to the hand, but not in contact with any surface, and the temperature inside the cylinder was kept at 60°C (range 55-65°C) by altering the thermostat on the water heater which was incorporated into the pump system ensuring circulation of the water.

In order to validate this new system we studied 8 patients who were undergoing elective cardiac catheterisation and who had an indwelling femoral artery sheath through which arterial samples could be obtained. After their investigation they were taken to the clinical investigation area where they placed their arm in the arm heater for 120 minutes. In order to obtain results over a range of glucose values, the subjects received a constant rate infusion of 20% glucose (360 mg/kg.hr) and insulin (50 mU/kg.hr) over the course of 120 minutes. “Arterialised” venous and true arterial samples were taken every 30 minutes or analysis of plasma glucose. Taking a mean of 40 samples, “arterialised” venous blood glucose levels were 1.9% lower than true arterial samples (Table 8). After 120 minutes, the mean “arterialised”

venous oxygen saturation was 2.3% lower than true arterial blood (Table 9). These results are very comparable to those achieved with other methods (Liu et al 1992).

Fibrinolytic parameters

Details of the commercial kits used for the measurement of tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor 1 (PAI-1) antigen and activity are given in Chapter 6.

TABLE 7. INSULIN PRIME DURING THE EUGLYCAEMIC CLAMP

Time (minutes)	Insulin infusion rate (mU/m² surface area min)
0-1	127.6
1-2	113.6
2-3	101.2
3-4	90.2
4-5	80.2
5-6	71.4
6-7	63.6
7-8	56.8
8-9	50.4
9-10	45.0
10-120	40.0

TABLE 8. PLASMA GLUCOSE CONCENTRATIONS IN 8 SUBJECTS

Time (min)	0	30	60	90	120
Femoral artery glucose (mmol/L)	5.8 (0.6)	10.8 (0.8)	11.9 (1.0)	12.6 (1.5)	11.5 (2.3)
“Arterialised” glucose (mmol/L)	5.7 (0.6)	10.5 (0.8)	11.8 (0.7)	12.2 (1.5)	11.4 (2.3)

Data are mean (SD)

TABLE 9. ARTERIAL AND “ARTERIALISED” VENOUS OXYGEN SATURATIONS

Subject	1	2	3	4	5	6	7	8	Mean (SD)
Femoral artery (%)	91	94	93	89	91	92	92	91	91.6 (1.5)
“Arterialised” (%)	88	93	89	89	87	88	91	89	89.3 (1.9)

CHAPTER 3

HYPERINSULINAEMIA IN ISCHAEMIC HEART DISEASE: THE IMPORTANCE OF MYOCARDIAL INFARCTION AND LEFT VENTRICULAR FUNCTION

3.1 SUMMARY

Elevated circulating insulin levels have been reported in ischaemic heart disease and may be of aetiological importance. Previous studies have not considered the potential influence of heart failure or of previous myocardial infarction as opposed to stable angina. I therefore measured the insulin response to a 75 g oral glucose tolerance test in 5 groups with normal glucose tolerance, comparing normal male controls to men with chronic stable angina, men with recent myocardial infarction assessed either at 3 weeks or 3 months after the acute event, and men with chronic severe heart failure. Only patients with chronic heart failure had fasting hyperinsulinaemia and this probably reflected the associated neuroendocrine abnormalities. Stimulated hyperinsulinaemia was present in all patient groups but was less pronounced and of shorter duration in patients with angina. At 120 minutes, only patients with heart failure or previous myocardial infarction were hyperinsulinaemic. The degree of stimulated hyperinsulinaemia was not influenced by the presence of heart failure or by the length of time from infarction. Hyperinsulinaemia is associated with impaired peripheral muscle glucose uptake and metabolism and might contribute to the development of muscular fatigue on exertion in patients with previous myocardial infarction or with heart failure.

3.2 INTRODUCTION

Hyperinsulinaemia has been described in obesity, impaired glucose tolerance (IGT), non-insulin dependent diabetes mellitus, hypertension and ischaemic heart disease (IHD) (Reaven 1988). Insulin may have a direct role in the development of atherosclerosis or may influence other risk factors for IHD, such as blood pressure and lipoproteins (Stout 1990). The relationship of fasting and stimulated hyperinsulinaemia to IHD has not been clearly defined. Previous studies in which an oral glucose load was given to patients with IHD have frequently reported stimulated hyperinsulinaemia (Peters and Hales 1965; Nikkila et al 1965; Tzagournis et al 1967; Tzagournis et al 1968; Kashyap et al 1970; Malherbe et al 1971; Gertler et al 1972; Berchthold et al 1972; Sorge et al 1976; Larsen et al 1981; Hamsten et al 1987a; Lichtenstein et al 1987), less often fasting hyperinsulinaemia (Peters and Hales 1965; Larsen et al 1981; Lichtenstein et al 1987) and two studies have failed to show fasting or stimulated hyperinsulinaemia (Bergstrand et al 1979; Jackson et al 1983). Earlier studies were limited as they frequently involved only patients with previous myocardial infarction and they failed to differentiate the importance of heart failure. Often patients with IGT or diabetes mellitus were not excluded (Nikkila et al 1965; Tzagournis et al 1967; Tzagournis et al 1968; Kashyap et al 1970; Malherbe et al 1971; Gertler et al 1972; Berchthold et al 1972; Sorge et al 1976; Hamsten et al 1987a; Lichtenstein et al 1987; Bergstrand et al 1979) and patients and controls were not matched for other important confounding variables including age, body mass index (BMI) or blood pressure (Peters and Hales 1965; Nikkila et al 1965;

Tzagournis et al 1967; Tzagournis et al 1968; Malherbe et al 1971; Gertler et al 1972; Berchthold et al 1972; Sorge et al 1976; Larsen et al 1981; Hamsten et al 1987a; Lichtenstein et al 1987; Bergstrand et al 1979; Jackson et al 1983). The administered glucose load has not been the standard 75 g in all studies and the subsequent blood sampling has sometimes been limited to 60 minutes.

The immunoreactive insulin (IRI) response to a 75 g oral glucose tolerance test was measured over 120 minutes in 5 groups with normal glucose tolerance matched for age, BMI and blood pressure. I have compared normal male controls to men with chronic stable angina, men with myocardial infarction assessed either at 3 weeks or 3 months after the acute event and men with severe chronic heart failure.

3.3 METHODS

Subjects

Normal control subjects (n = 22) were identified at random from the Lothian Health Board register and they had no previous history of hypertension, IHD or family history of diabetes mellitus. They were taking no regular medication and had a normal 12 lead electrocardiogram. Patients with stable angina (n = 15) were identified on the basis of a typical history of exertional chest pain and the development of significant electrocardiographic changes (> 1 mV ST segment depression 0.06 seconds after the J point) during treadmill exercise testing. None had any clinical evidence of heart failure. At the time of study they were receiving nifedipine and none were taking β blockers. The 2 groups of patients with recent myocardial infarction had an initial diagnosis based upon World Health Organisation

criteria. One group was studied 3 weeks after the acute event (MI Group I, $n = 26$) and one group was studied 12 weeks after the acute event (MI Group II, $n = 15$). None of these patients had symptomatic heart failure and all were taking aspirin. Twenty (77%) of the patients in MI Group I and all those in MI Group II were taking a cardioselective β blocker. Patients with chronic heart failure ($n = 16$) were free of oedema but remained severely restricted (all NYHA grade III). At the time of study the heart failure group were all receiving a loop diuretic with potassium supplements and none were taking an angiotensin converting enzyme inhibitor.

I screened 93 patients attending the out patient department with established IHD and with fasting plasma glucose < 6.7 mmol/L using a 75 g oral glucose tolerance test. Twenty-one (23%) patients were found to have IGT or diabetes mellitus according to World Health Organisation criteria (2 hour glucose > 11 mmol/L = diabetes, > 7.8 mmol/L = IGT) (World Health Organisation 1985) and were excluded from this analysis because both of these conditions have previously been associated with hyperinsulinaemia (Reaven 1988).

Data from 72 patients and 22 normal controls are presented. Details of age, BMI (weight/height²), blood pressure, smoking habit and left ventricular ejection fraction are given in Table 10. The 5 groups were well matched for age, BMI and blood pressure. Ten (63%) of the patients with chronic heart failure were smokers compared to less than 10% in the other groups. Measurement of left ventricular function was made by radionuclide ventriculogram in patients with a history of myocardial infarction or heart failure. MI Group I and MI Group II had similar and well preserved left ventricular function, whereas the group with NYHA grade III

heart failure had significantly decreased left ventricular ejection fraction by comparison with those groups.

The study received the approval of the Lothian Health Board Ethical Committee for medical research.

Oral glucose tolerance test

Patients and controls attended the department after a 12 hour overnight fast. An indwelling 16 G intravenous cannula was inserted into an antecubital vein and they rested for 30 minutes. After a baseline blood sample they took 75 g glucose monohydrate in 200 mL water. Further blood samples were taken at 30, 60, 90 and 120 minutes. Samples were placed on ice and plasma separated within one hour and stored at -40°C. Each sample was assayed for glucose and IRI. Glucose was measured using a Cobas Bio analyser by the hexokinase method (Schmidt 1973) with an interassay coefficient of variation of 1.7%. IRI was measured by radioimmunoassay (Soeldner and Slone 1965) with a coefficient of variation of 6.8 to 7.5%. This assay does not differentiate between insulin, proinsulin and proinsulin split-products.

Statistical analysis

Descriptive data are expressed as mean (SEM). Areas under the curve (AUC) were calculated using the trapezium rule. Glucose values are expressed as arithmetic mean (95% CI of mean). Values for insulin and insulin/glucose ratio were compared after logarithmic transformation and are expressed as geometric mean (95% CI of mean). Analysis of variance (ANOVA) was used to assess differences among the means of the groups. Where ANOVA revealed a significant difference between the

5 groups, further pairwise comparisons were made using Fisher's least-significant-difference test. Data was stored on a Dell 486P/33 personal computer and statistical analyses were performed using SYSTAT for Windows, Version 5 (Systat Inc. 1992, Evanston, Illinois). The level of significance was taken to be $p < 0.05$.

3.4 RESULTS (Table 11)

Glucose response to oral glucose (Figure 1)

There was no significant difference between the 5 groups for glucose levels either fasting, or at 120 minutes or AUC. Despite all patients having normal glucose tolerance, glucose levels at 120 minutes tended to be higher compared to controls in patients with previous MI or heart failure.

Insulin response to oral glucose (Figure 2)

Plasma insulin levels were significantly different between the 5 groups for fasting ($p = 0.012$), at 120 minutes ($p = 0.002$) and for AUC ($p = 0.011$). Follow up pairwise comparisons for fasting insulin showed significant differences between patients with heart failure v normal controls ($p = 0.002$), v MI Group I ($p = 0.006$), and v MI Group II ($p = 0.003$), and borderline significant difference between patients with heart failure v stable angina ($p = 0.06$). Follow up pairwise comparisons for 120 minute levels showed significant differences between normal controls v MI Group I ($p = 0.001$), v MI Group II ($p = 0.02$), and v chronic heart failure ($p = 0.001$). In addition, patients with stable angina were significantly different from MI Group I ($p = 0.03$) and from chronic heart failure ($p = 0.02$). Follow up pairwise comparisons for AUC showed significant differences between normal controls and all patient

groups, controls v stable angina ($p = 0.03$), v MI Group I ($p = 0.005$), v MI Group II ($p = 0.01$), and v chronic heart failure $p = 0.001$, but there were no significant differences between patient groups.

Insulin/glucose ratio response to oral glucose (Figure 3)

The ratio of plasma insulin/glucose was significantly different between the 5 groups for fasting ($p = 0.007$), at 120 minutes ($p = 0.004$) and for AUC ($p = 0.004$). Pairwise comparisons for fasting ratios showed significant differences between patients with heart failure v normal controls ($p = 0.001$), v stable angina ($p = 0.04$), v MI Group I ($p = 0.005$) and v MI Group II ($p = 0.001$). Pairwise comparisons revealed significant differences for values at 120 minutes between normal controls v MI Group I ($p = 0.0012$), v MI Group II ($p = 0.03$) and v chronic heart failure ($p = 0.001$), and between stable angina v chronic heart failure ($p = 0.03$). Pairwise comparisons between the groups for AUC showed significant differences between normal controls v stable angina ($p = 0.05$), v MI Group I ($p = 0.003$), v MI Group II ($p = 0.005$) and v chronic heart failure ($p < 0.001$).

3.5 DISCUSSION

The most important finding of this study was to suggest that the presence and nature of hyperinsulinaemia in patients with IHD may be crucially influenced by previous myocardial infarction or the extent of left ventricular dysfunction. Unlike previous studies (Nikkila et al 1965; Tzagournis et al 1967; Tzagournis et al 1968; Kashyap et al 1970; Malherbe et al 1971; Gertler et al 1972; Berchthold et al 1972;

Sorge et al 1976; Larsen et al 1981; Hamsten et al 1987a; Lichtenstein et al 1987; Bergstrand et al 1979; Jackson et al 1983) I have taken care to minimise the influence of other conditions known to influence insulin sensitivity by excluding patients with impaired or abnormal glucose tolerance and by matching patient groups and controls for age, BMI and blood pressure (Reaven 1988). Fasting hyperinsulinaemia was only a feature of patients with chronic heart failure. Stimulated hyperinsulinaemia was present in chronic stable angina, for at least 3 months following recent acute myocardial infarction without clinically significant left ventricular impairment, and in chronic heart failure. Although patients with angina or recent infarction had similar fasting levels of insulin, at 120 minutes patients with recent infarction had persistently elevated insulin, similar in magnitude to that of the heart failure group and significantly higher than controls, whilst at 120 minutes patients with angina did not differ significantly from controls but were significantly lower than MI group I or patients with chronic heart failure. It may be that the persistent elevation of insulin at 120 minutes in those with recent myocardial infarction, as opposed to a return to normal insulin levels in those with stable angina, is associated with an increased tendency to occlusive thrombus formation in addition to atheroma. This hypothesis is supported by evidence linking hyperinsulinaemia to impaired endogenous fibrinolysis (Juhan-Vague et al 1991). Alternatively, stimulated hyperinsulinaemia may occur as a response to the presence of left ventricular dysfunction, irrespective of the presence of symptoms. If this were the case, then the degree of the stimulated response is not critically dependent upon the extent of ventricular impairment, as the 120 minute and AUC values were similar in those with a well preserved left

ventricular ejection fraction and those with severely impaired ejection fraction. The degree of stimulated hyperinsulinaemia in those with recent myocardial infarction did not differ between Group I, studied 3 weeks after the acute event, and Group II, studied 3 months after. This implies that, in the absence of heart failure, the acute phase response to acute myocardial infarction does not influence hyperinsulinaemia after 3 weeks from the time of infarction. It is impossible to say whether stimulated hyperinsulinaemia was present prior to infarction or developed as a consequence. Three large longitudinal population studies have suggested that hyperinsulinaemia is a risk factor for subsequent myocardial infarction (Welborn et al 1979; Ducimetiere et al 1980; Pyorala et al 1985). My data would support this hypothesis but would also suggest that fasting hyperinsulinaemia is a late development confined to patients with moderate or severe heart failure.

No previous study has specifically addressed the importance of insulin in heart failure secondary to ischaemic heart disease but low fasting insulin levels and an impaired response to intravenous tolbutamide were found in a previous study in 8 patients with heart failure secondary to rheumatic heart disease (Sharma et al 1970). The effect of an oral glucose load was not tested and the patients studied were severely ill and bedridden despite treatment with digoxin and diuretics. In a study of 8 patients with congestive heart failure (Paolisso et al 1991), of whom only one had ischaemic heart disease, impaired insulin sensitivity was shown to be a feature and the degree of insulin resistance appeared to correlate positively with plasma noradrenaline concentrations. In end-stage disease it is conceivable that pancreatic hypoperfusion might lead to reduced insulin secretion, but in less severe heart failure

there are a number of possible influences on insulin action which might lead to insulin resistance and hyperinsulinaemia. The neuroendocrine response in heart failure leads to increased secretion of catecholamines, cortisol and growth hormone (Bayliss et al 1987) all of which antagonise the peripheral action of insulin. Physical inactivity is associated with hyperinsulinaemia and insulin resistance and this may be of relevance to patients with ischaemic heart disease and particularly those with heart failure (Rosenthal et al 1983). Although there is no evidence from studies in man to support a primary role for hyperinsulinaemia or insulin resistance in the development of heart failure, impaired ventricular function associated with prolonged insulin resistance has recently been described in a rat model of streptozocin-induced diabetes (Schaffer et al 1993). This would be of particular importance because of evidence that angiotensin converting enzyme inhibitors may favourably modify insulin resistance (Pollare et al 1989b).

Hyperinsulinaemia is an important finding in IHD because it is associated with dyslipidaemia, hypertension, impaired fibrinolysis and sodium retention by the kidney (Reaven 1988; Stout 1990). In addition, hyperinsulinaemia, particularly in the presence of normal glucose levels, suggests a defect in utilisation of substrate by peripheral muscle, as has been shown in diabetes mellitus (Shulman et al 1990), and this may be relevant to the symptoms of fatigue and diminished effort tolerance which are prevalent in patients with heart failure or after myocardial infarction. Secondly, studies in patients with hypertension have illustrated the potential adverse effects of β blockers (Pollare et al 1989a) and beneficial effects of angiotensin

converting enzyme inhibitors on insulin resistance (Pollare et al 1989b) and these are classes of drug widely prescribed to patients with IHD.

Limitations of this study

It should be noted that nearly all current insulin immunoassays cross react with proinsulin and 32-33 split proinsulin and these molecules may form a considerable part of total insulin immunoreactivity after a glucose stimulus in subjects with non-insulin dependent diabetes mellitus (Sobey et al 1989; Clark et al 1992). However, there is no information to suggest that insulin precursor levels are increased in non-diabetic subjects with IHD, and even amongst those with non-insulin dependent diabetes, it appears that those with mild or diet controlled disease do not show excess secretion of insulin precursor molecules (Clark et al 1992). The stimulated hyperinsulinaemia in some groups may in part reflect relative hyperglycaemia. The elevated ratio of insulin/glucose in those with heart failure or recent myocardial infarction does not support this, however, and suggests that the available insulin was not acting normally to increase glucose uptake, which indicates the presence of insulin resistance.

Although there are no studies of the effect of chronic β blockade on insulin resistance in patients with IHD, in patients with hypertension β blockers have been associated with impaired insulin sensitivity and hyperinsulinaemia (Pollare et al 1989a). In this study, the groups taking β blockers were those with a recent history of myocardial infarction and without heart failure. In MI Group I and MI Group II, fasting levels of insulin were similar to both normal controls and men with stable angina who were not taking a β blocker. Stimulated levels of insulin in MI Group I

and MI Group II were similar to those with heart failure who were not taking a β blocker. This suggests that β blockers were not responsible for hyperinsulinaemia in this study.

All of the patients in the group with chronic heart failure were taking loop diuretics with potassium supplements and there are case reports of these being associated with IGT (Toivenen et al 1966). However, the proposed mechanism is not through increased insulin release but through inhibition of insulin secretion and this has been confirmed in an animal study (Sandstrom and Semlin 1988). In studies which have examined the effect of frusemide on glucose tolerance and insulin response in patients with hypertension (Jackson and Nellen 1966) and following myocardial infarction (Efendic et al 1984) there was no effect on fasting or stimulated insulin.

In this study the group of patients with heart failure was the only group to contain a high proportion of smokers (10/16) and cigarette smoking has been associated in normal volunteers with stimulated hyperinsulinaemia in response to an OGTT and increased insulin resistance (Facchini et al 1992). However, a comparison of the smokers and non-smokers in the heart failure group (Table 12) showed no significant difference between smokers and non-smokers. This is also supported by data from the Helsinki Policeman Study in which 426 of 982 men were smokers and in whom there was no difference in fasting or stimulated insulin response (Pyorala et al 1985). Unpublished data from the Edinburgh-Stockholm Study also failed to find any difference in insulin response between smokers and non-smokers (Dr RA Riemersma, personal communication).

3.6 CONCLUSION

In patients with ischaemic heart disease, fasting hyperinsulinaemia is only present in severe heart failure, whereas stimulated hyperinsulinaemia is prominent amongst those with previous myocardial infarction and those with heart failure, and is also present, but less marked, in those with chronic stable angina.

TABLE 10. BASELINE CHARACTERISTICS OF THE 5 GROUPS

	Controls (n=22)	Stable Angina (n=15)	MI Group I (n=26)	MI Group II (n=15)	Heart Failure (n=16)
Age (years)	56 (1)	60 (2)	61 (2)	63 (2)	60 (2)
BMI (kg/m ²)	24.9 (0.6)	26.7 (0.8)	25.3 (0.6)	25.2 (0.7)	25.3 (0.7)
Systolic BP	118 (4)	127 (5)	122 (4)	127 (5)	127 (5)
Diastolic BP	79 (2)	78 (3)	76 (2)	80 (3)	80 (3)
Smokers	1	1	2	1	10
LVEF	NM	NM	0.37 (0.02)	0.39 (0.02)	0.20 ⁺ (0.02)

MI = myocardial infarction, BMI = body mass index, LVEF = left ventricular ejection fraction, NM = not measured, Values are mean (SEM) where applicable

⁺p < 0.001; Student's *t* test v both of the MI groups

TABLE 11. PLASMA GLUCOSE AND INSULIN RESPONSES TO A 75 G ORAL GLUCOSE TOLERANCE TEST IN NORMAL CONTROLS AND PATIENTS WITH STABLE ANGINA, PATIENTS AFTER ACUTE MYOCARDIAL INFARCTION STUDIED AT 3 WEEKS OR 3 MONTHS, AND PATIENTS WITH CHRONIC HEART FAILURE

	Controls (n=22)	Stable Angina (n=15)	MI Group I (n=26)	MI Group II (n=15)	Heart Failure (n=16)	ANOVA p value
Fasting						
Glucose (mmol/L)	5.0 (4.8 to 5.2)	5.1 (5.0 to 5.3)	5.0 (4.8 to 5.2)	5.3 (5.0 to 5.5)	5.0 (4.7 to 5.4)	0.483
Insulin (mU/L)	5.5 (4.3 to 7.0)	7.2 (5.0 to 10.2)	6.2 (5.1 to 7.5)	5.4 (3.5 to 8.1)	11.5 (7.1 to 18.8)	0.012
Insulin/Glucose (mU/mmol)	1.1 (0.8 to 1.4)	1.4 (1.0 to 2.0)	1.2 (1.0 to 1.5)	1.0 (0.7 to 1.5)	2.3 (1.4 to 3.7)	0.007
120 minute						
Glucose (mmol/L)	5.3 (4.8 to 5.8)	5.1 (4.6 to 5.7)	5.9 (5.4 to 6.4)	6.1 (5.4 to 6.8)	6.0 (5.4 to 6.6)	0.076
Insulin (mU/L)	30.8 (22.0 to 43.2)	36.9 (27.8 to 49.0)	62.2 (46.8 to 82.6)	56.5 (37.2 to 85.7)	69.1 (47.9 to 99.8)	0.002
Insulin/Glucose (mU/mmol)	5.9 (4.4 to 7.8)	7.3 (5.8 to 9.2)	10.8 (8.5 to 13.7)	9.5 (6.6 to 13.9)	11.9 (8.6 to 16.3)	0.004
AUC						
Glucose (min.mmol/L)	791 (728 to 855)	894 (823 to 966)	848 (804 to 892)	846 (787 to 906)	837 (758 to 915)	0.256
Insulin (min.mU/L)	5041 (3985 to 6378)	7377 (6088 to 8939)	7951 (6396 to 9884)	8050 (5947 to 10898)	8998 (6906 to 11721)	0.011
Insulin/Glucose (min.mU/mmol)	6.1 (5.0 to 7.5)	8.4 (7.1 to 9.8)	9.4 (7.7 to 11.6)	9.6 (7.3 to 12.6)	10.9 (8.8 to 13.6)	0.004

MI = myocardial infarction, AUC = Area under the curve calculated from samples at 0, 30, 60, 90 and 120 minutes, ANOVA = analysis of variance between the 5 groups. Values are arithmetic mean for glucose and geometric mean for insulin and insulin/glucose ratio, with the 95% CI for the means in parentheses

TABLE 12. PLASMA INSULIN RESPONSE TO A 75 G ORAL GLUCOSE TOLERANCE TEST IN PATIENTS WITH CHRONIC HEART FAILURE - SMOKERS V NON-SMOKERS

Time (min)	Smokers (n=10)	Non-smokers (n=6)
0	10.5 (4.8 to 23.1)	12.2 (6.4 to 23.3)
30	72.5 (47.9 to 108.9)	61.4 (38.1 to 98.5)
60	89.1 (61.0 to 130.3)	96.7 (67.4 to 138.4)
90	91.9 (55.2 to 153.0)	78.2 (47.5 to 129.0)
120	98.5 (68.7 to 139.8)	56.0 (33.5 to 93.6)

Plasma insulin in mU/L

Values are geometric mean (95% CI)

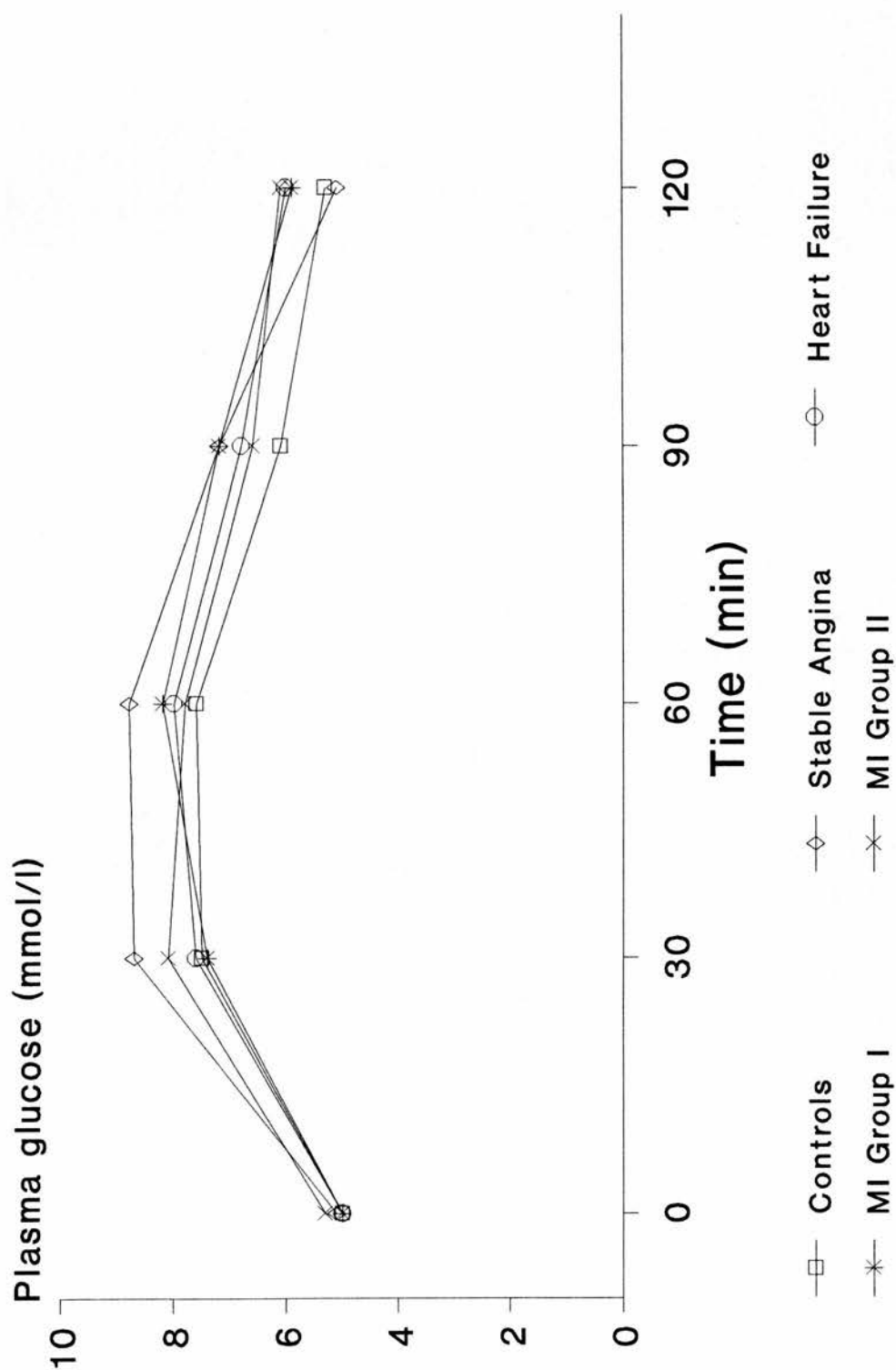


Figure 1. Arithmetic mean plasma glucose concentrations before and after a 75 g oral glucose challenge in the 5 groups

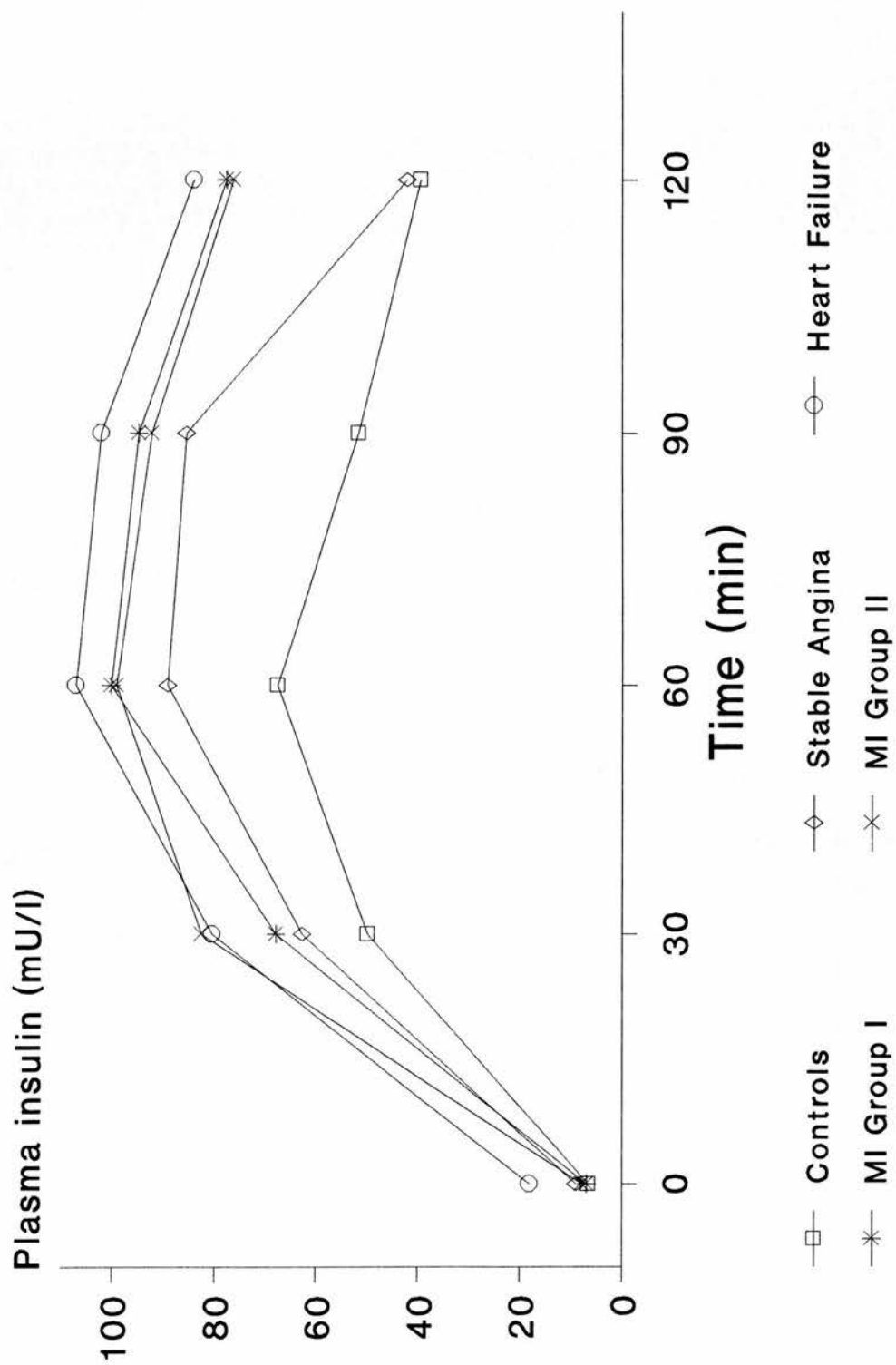


Figure 2. Arithmetic mean plasma insulin concentrations before and after a 75 g oral glucose challenge in the 5 groups

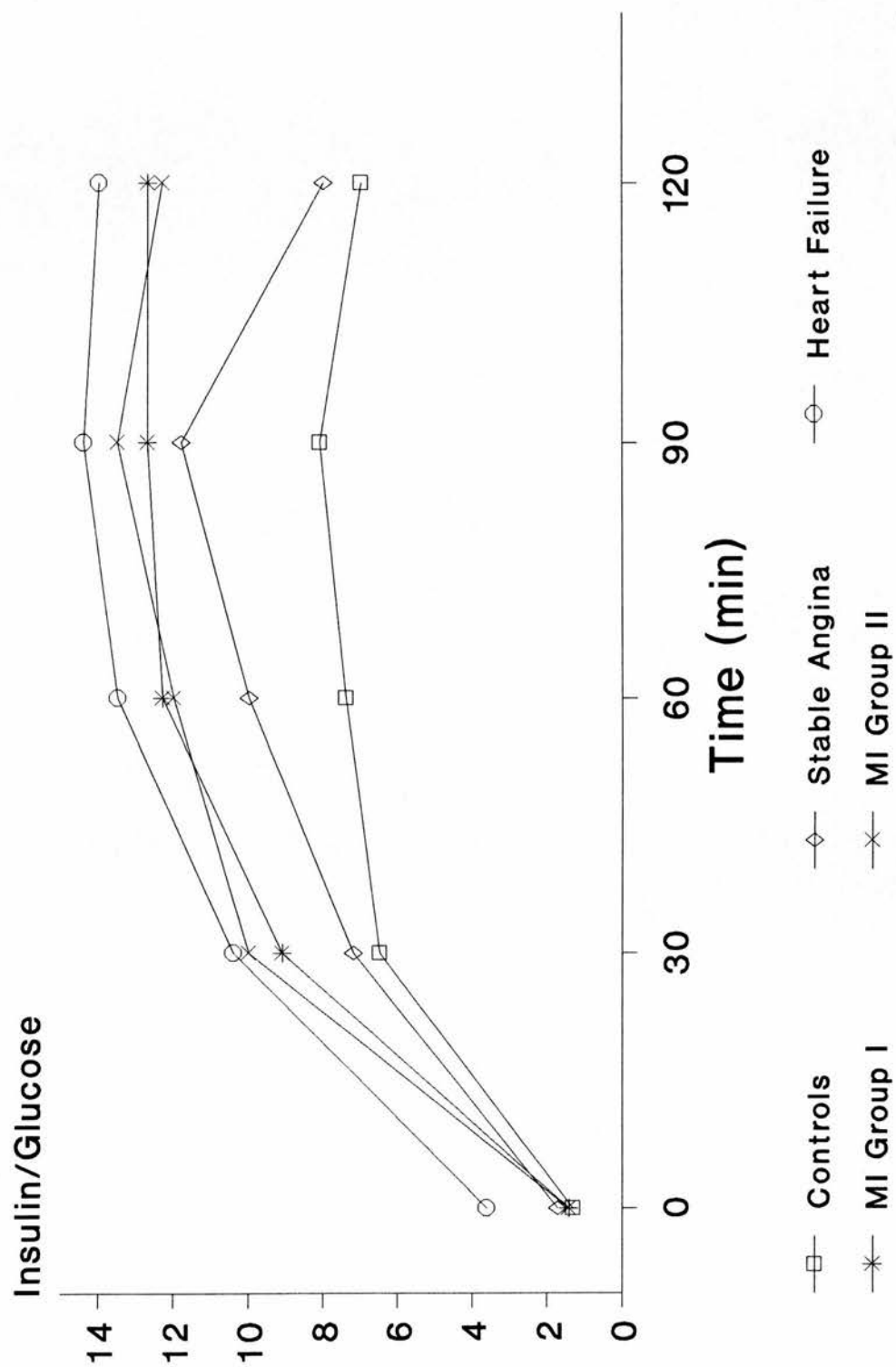


Figure 3. Arithmetic mean of plasma glucose/insulin ratio before and after a 75 g oral glucose challenge in the 5 groups

CHAPTER 4

IMPAIRED GLUCOSE TOLERANCE, HYPERINSULINAEMIA AND INSULIN RESISTANCE IN PATIENTS WITH ISCHAEMIC HEART DISEASE

4.1 SUMMARY

To compare the extent of hyperinsulinaemia during a 75 g oral glucose tolerance test in ischaemic heart disease patients with either normal or impaired glucose tolerance, 72 patients with normal glucose tolerance and 18 with impaired glucose tolerance were compared. Patients with impaired glucose tolerance had both fasting ($11.6 \text{ v } 7.2 \text{ mU/L}$, $p = 0.04$) and stimulated (120 minute insulin, $118.3 \text{ v } 55.5 \text{ mU/L}$, $p < 0.001$) hyperinsulinaemia.

A subgroup of 56 patients also underwent radionuclide ventriculography. There was a significant correlation between left ventricular ejection fraction and fasting insulin ($r = -0.392$, $p = 0.003$), but there was no relationship with the stimulated insulin response. Left ventricular ejection fraction, body mass index and fasting glucose were entered into a multiple linear regression model with fasting insulin as the dependent variable. Left ventricular ejection fraction remained a significant predictor of fasting plasma insulin.

The prevalence of impaired glucose tolerance amongst non-diabetic men with ischaemic heart disease was 20%. Both fasting and stimulated hyperinsulinaemia were significantly greater in patients with impaired glucose tolerance. In a subgroup of 56 patients, there was an inverse relationship between left ventricular ejection fraction and fasting insulin concentration, but there was no relationship between ejection fraction and glycaemia or stimulated hyperinsulinaemia.

4.2 INTRODUCTION

Hyperinsulinaemia has been described in patients with ischaemic heart disease (Peters and Hales 1965) and is also known to occur in obesity, impaired glucose tolerance, non-insulin dependent diabetes mellitus and hypertension (Reaven 1988). Insulin may have a direct role in the development of atherosclerosis, or may influence other risk factors for ischaemic heart disease, such as blood pressure, lipoproteins and endogenous fibrinolysis (Stout 1990; Savage 1993). Impaired glucose tolerance was previously known as "borderline diabetes," and the current definition was produced by the World Health Organisation in 1985. Impaired glucose tolerance is an intermediate state in which glucose tolerance is abnormal but there is no risk of the microvascular complications of diabetes, although the risk of subsequently developing non-insulin dependent diabetes is increased (Yudkin et al 1990). Impaired glucose tolerance is, however, a risk factor for developing macrovascular disease in subjects without known cardiac disease (Fuller et al 1980; Fontbonne et al 1989; McKeigue et al 1993) and is also more prevalent amongst young patients with myocardial infarction than matched controls (Hamsten et al 1987a). Hyperinsulinaemia and insulin resistance are features of impaired glucose tolerance in subjects who are otherwise healthy (Reaven et al 1989). It has been suggested that impaired glucose tolerance and obesity only convey independent excess risk of cardiovascular disease in men who are hyperinsulinaemic (Modan et al 1991). Previous studies in selected groups of patients with ischaemic heart disease in which the results for those with abnormal glucose tolerance were described

separately have shown conflicting results regarding the presence of fasting or stimulated hyperinsulinaemia (Hamsten et al 1987a; Gertler et al 1972; Sorge et al 1976; Efendic et al 1984) but these studies were carried out prior to the recent and widely accepted definition of impaired glucose tolerance (World Health Organisation, 1985) and generally with insulin assays of poor specificity.

The purpose of this study was to examine the immunoreactive insulin response to a 75 g oral glucose tolerance test amongst a broad range of patients with ischaemic heart disease and compare those with impaired glucose tolerance (120 minute plasma glucose ≥ 7.8 and ≤ 11.0 mmol/L) to those with normal glucose tolerance (120 minute glucose < 7.8 mmol/L).

4.3 METHODS

Patients

Ninety-three patients attending the Department of Cardiology as out patients and with a principal diagnosis of ischaemic heart disease who were in a stable condition and who gave their informed consent to undergo an oral glucose tolerance test were recruited. The study received the approval of the Lothian Health Board Ethical Committee for medical research. The diagnosis of ischaemic heart disease was based upon a history of previous myocardial infarction meeting World Health Organisation criteria or a combination of a history of typical stable angina pectoris and recent positive exercise tolerance test (≥ 1 mV ST segment depression 0.06 seconds after the J point in at least one of 12 standard electrocardiographic leads). All patients had a fasting plasma glucose of less than 6.7 mmol/L and none

were suspected of having abnormal glucose tolerance. Patients with diabetes mellitus or with a history of diabetes in a first degree relative were excluded from this study. A formal measurement of left ventricular function by radionuclide angiography was made as part of the routine assessment of 56 (70%) patients.

Oral glucose tolerance test

Subjects attended the department at 08.00 hours after a 12 hour overnight fast. An indwelling 16 G intravenous cannula was inserted into an antecubital vein, and they rested sitting for 30 minutes. Prior to blood sampling on each occasion 5 mL of blood was withdrawn and discarded. After a baseline blood sample they took 75 g glucose monohydrate in 200 mL water. Further blood samples were taken at 30, 60, 90 and 120 minutes. Samples were placed immediately onto melting ice. Plasma was separated within 1 hour, and stored at -40°C. Each sample was assayed for immunoreactive insulin. Glucose was measured using a Cobas Bio analyser by the hexokinase method (Schmidt 1973) with an interassay coefficient of variation of 1.7%. Immunoreactive insulin was measured by radioimmunoassay (Soeldner and Slone 1965) with a coefficient of variation of 6.8-7.5%. This assay shows less than 20% cross-reactivity with proinsulin and 32,33 split proinsulin. This means that even if proinsulin and its split products were present in 30% of the molar concentration of insulin they would represent less than 6% of the measured immunoreactive insulin. In a recent study of subjects with impaired glucose tolerance, these molecules were found to represent only 15% of the total immunoreactive insulin measured by the assay (Davies et al 1993).

Statistical analysis

Descriptive data are given as mean (SD). Areas under the curve were calculated using the trapezium rule. Body mass index was calculated as weight/height².

Glucose values are given as the arithmetic mean (95% CI of mean). Values for insulin and insulin/glucose ratio were compared after logarithmic transformation to normalise their distribution, and are expressed as the geometric mean (95% CI of mean). The data have been expressed in this form (mean [95% CI of mean]) because a consequence of the need for logarithmic transformation of insulin data is that CI of the difference between the impaired glucose tolerance and normal glucose tolerance groups represent CI of the ratio between groups rather than absolute values (Gardner and Altman 1989). Statistical testing of the differences between the 2 groups was by Student's t test for unpaired observations assuming unequal variances. Pearson linear correlation coefficients and multiple linear regression were performed using SYSTAT for Windows Version 5 (Systat Inc. 1992, Evanston, Illinois). The two-tailed level of significance was $p < 0.05$.

4.4 RESULTS

Baseline characteristics

The clinical characteristics of the 2 groups are shown in Table 13. There were no significant differences between the 2 groups in their baseline characteristics and in particular the groups were well matched for previous myocardial infarction, heart failure and the prescription of drug therapy.

Oral glucose tolerance test

(A summary of the principal results is shown in Table 14)

Fasting plasma glucose did not differ between the 2 groups. The expected response (by definition) to the oral glucose load is shown in Figure 4.

Fasting insulin concentrations were significantly higher in the impaired glucose tolerance group ($p = 0.044$). Figure 4 shows that insulin levels were persistently higher throughout the test for the impaired glucose tolerance group with no evidence of a failure of early insulin release in those with impaired glucose tolerance. There were significant differences between the 2 groups for both area under the curve ($p = 0.022$) and 120 minute insulin ($p < 0.001$).

The influence of left ventricular function on insulin and glucose in ischaemic heart disease

In order to gain further information about the possible influence of left ventricular function on glycaemia and hyperinsulinaemia in patients with ischaemic heart disease, I considered the 56 patients (44 with normal glucose tolerance, 12 with impaired glucose tolerance) in whom measurement of left ventricular function had also been made. Table 15 shows a Pearson correlation matrix for these 56 patients including both fasting and 120 minute glucose and insulin concentration with body mass index and left ventricular ejection fraction. There were no significant correlations between glucose concentration and either body mass index or left ventricular ejection fraction. There were significant correlations between fasting insulin and body mass index, 120 minute insulin and ejection fraction (in descending order of significance) but not with fasting glucose. Figure 5 shows a plot of log

fasting insulin against left ventricular ejection fraction and Figure 6 a plot of log fasting insulin against body mass index. There were significant correlations between 120 minute insulin and 120 minute glucose, fasting insulin and body mass index (in descending order of significance) but not with left ventricular ejection fraction. Table 16 shows the results of a multiple regression model with fasting insulin as the dependent variable and including fasting glucose, body mass index and left ventricular ejection fraction as independent variables; both body mass index ($p < 0.001$) and left ventricular ejection fraction ($p = 0.013$) were significant predictors. Table 17 shows the results of a multiple regression model with 120 minute insulin as the dependent variable and including body mass index, left ventricular ejection fraction, 120 minute glucose and fasting glucose and insulin; only 120 minute glucose ($p < 0.001$) and fasting insulin ($p = 0.008$) remained significant predictors.

4.5 DISCUSSION

This study showed that impaired glucose tolerance was present in 20% of patients with ischaemic heart disease who were not suspected of abnormal glucose tolerance. Impaired glucose tolerance was associated with significant fasting and stimulated hyperinsulinaemia over and above that which occurs in patients with ischaemic heart disease who have normal glucose tolerance. The results also showed that body mass index and left ventricular ejection fraction were important

determinants of fasting insulin concentrations but did not have a major influence on either glycaemia or the stimulated insulin response.

These results confirm previous observations in healthy subjects that circulating insulin levels are increased in impaired glucose tolerance compared to normal glucose tolerance (Reaven et al 1989). This was also suggested in earlier studies in selected patients with ischaemic heart disease which used differing definitions of abnormal glucose tolerance (Hamsten et al 1987a; Gertler et al 1972; Sorge et al 1976) although one study reported a diminished insulin response to an intravenous glucose tolerance test in patients who had abnormal glucose tolerance following myocardial infarction (Efendic et al 1984). In my study there was no evidence of a diminished early insulin response in patients with impaired glucose tolerance as has recently been suggested in otherwise healthy subjects with impaired glucose tolerance (Mitrakou et al 1992; Davies et al 1993). The relative specificity of the insulin assay in Newcastle suggests that for true hypoinsulinaemia to be present at 30 minutes, proinsulin and its split products would have had to contribute well over 50% of total insulin immunoreactivity. Recent results from healthy subjects with impaired glucose tolerance have suggested that the proportion of fasting proinsulin-like molecules is 15% compared to 12% in subjects with normal glucose tolerance (Davies et al 1993) and therefore it is unlikely that they represented a significant part of the difference in immunoreactive insulin levels found in my study. There is no information on insulin precursor levels in non-diabetic patients with ischaemic heart disease. The absence of a failure of early insulin secretion suggests that in patients with ischaemic heart disease the persistent hyperglycaemia of

impaired glucose tolerance is primarily due to reduced peripheral insulin sensitivity. Reduced insulin-mediated glucose uptake, measured by the insulin suppression test, has recently been shown in a group of men with ischaemic heart disease in a study which included patients with impaired glucose tolerance but did not differentiate their results (Young et al 1993). My results in the basal and stimulated state suggest that peripheral insulin resistance is greater in ischaemic heart disease patients with impaired glucose tolerance than normal glucose tolerance, and that frank deterioration to diabetic glucose tolerance is prevented by a compensatory increase in insulin secretion. This may well be relevant to prognosis in ischaemic heart disease, since hyperinsulinaemia has been associated with several important risk factors (Stout 1990; Savage 1993) and with increased cardiovascular morbidity in three large prospective studies (Welborn et al 1979; Ducimetiere et al 1980; Pyorala 1985). There is little information on the prognostic importance of impaired glucose tolerance or hyperinsulinaemia for patients with established ischaemic heart disease. Studies in patients with acute myocardial infarction have shown that elevated glucose levels on admission may predict pre-existing diabetes (Husband et al 1983) and a poorer prognosis (Oswald et al 1984; Oswald et al 1986; Bellodi et al 1989). However, glucose concentrations on admission reflect in part the neurohumoral disturbance associated with infarction, and this is proportional to the extent of left ventricular damage (Oswald et al 1986). The longer term risk due to atherothrombosis, rather than left ventricular dysfunction, may be more accurately predicted by risk factor assessment in the convalescent period. None of the above studies included a measurement of insulin.

Another important consequence of my study is to emphasise that when describing hyperinsulinaemia and insulin resistance in ischaemic heart disease it is crucial to fully define the glucose tolerance status of subjects (World Health Organisation 1985).

The analysis of patients with ischaemic heart disease who had normal glucose tolerance has suggested that fasting hyperinsulinaemia is most prominent in patients with heart failure (Chapter 3). This is supported by the inverse correlation between fasting insulin and left ventricular ejection fraction found in the combined groups. Insulin resistance in non-ischaemic heart failure has been ascribed to the neurohumoral response to heart failure (Paolisso et al 1991) much of which would be expected to antagonise insulin action. My own results suggest that impaired left ventricular function is an important determinant of fasting, but not stimulated, hyperinsulinaemia. This is consistent with observations that there is skeletal muscle metabolic dysfunction in heart failure (Wilson and Mancini 1993) and that muscle blood flow is an important determinant of whole body insulin sensitivity (Ganrot 1993). This study has also emphasised the recognised importance of body mass index in determining insulin levels (Reaven 1988).

In patients with normal glucose tolerance 120 minute insulin concentrations were elevated in those with prior myocardial infarction or chronic heart failure (Chapter 3). It is noteworthy that 120 minute insulin levels were considerably increased in patients with impaired glucose tolerance in this comparison, despite the groups being matched for the presence of heart failure and for previous myocardial infarction. It is impossible to say to what degree the stimulated hyperinsulinaemia

was due to higher glucose concentrations or to impaired insulin action. There was no relationship between stimulated insulin response and left ventricular ejection fraction in this study and a much weaker relationship with body mass index than for fasting insulin. This suggests that the influences on basal insulin secretion and the insulin response to glucose stimulation are different, which may be important if the risk of myocardial infarction is associated more with the stimulated insulin response.

Study limitations

It is well established from studies in hypertensive patients that glucose and insulin metabolism may be altered by both β blockers (Pollare et al 1989a) and angiotensin converting enzyme inhibitors (Pollare et al 1989b). The 2 groups in this study contained similar proportions of patients receiving these drugs, but I cannot exclude the possibility that the glucose or insulin response of some subjects was altered by their medication. However, no subject had undergone a change in their regular medication in the 4 week period prior to this study.

4.6 CONCLUSION

Impaired glucose tolerance was present in 20% of non-diabetic patients with ischaemic heart disease, none of whom were suspected of having abnormal glucose tolerance. Compared to patients with normal glucose tolerance, those with impaired glucose tolerance had significant fasting hyperinsulinaemia and significant stimulated hyperinsulinaemia in response to a 75 g oral glucose load. Fasting insulin concentrations were related to both body mass index and left ventricular ejection fraction, but these parameters did not significantly influence stimulated hyperinsulinaemia or glycaemia.

TABLE 13. CLINICAL DETAILS OF THE SUBJECTS

Characteristic	Normal Glucose Tolerance (n=72)	Impaired Glucose Tolerance (n=18)
Age (years)	61 (8)	57 (10)
Previous hypertension	6 (8%)	3 (17%)
Previous myocardial infarction	58 (81%)	15 (83%)
Chronic heart failure	18 (25%)	5 (28%)
Never smoked	20 (28%)	5 (28%)
Ex-smoker	35 (49%)	9 (50%)
Current smoker	17 (24%)	4 (22%)
β blocker	33 (46%)	9 (50%)
ACE inhibitor	9 (13%)	3 (17%)
Loop diuretic	18 (25%)	5 (28%)
Body mass index (kg/m²)	25.6 (3.0)	27.2 (3.4)
Systolic BP (mm Hg)	125 (21)	125 (17)
Diastolic BP (mm Hg)	78 (12)	79 (13)
Left ventricular ejection fraction (%)	33 (12) [n=44]	34 (10) [n=12]
Total cholesterol (mmol/L)	6.2 (1.2)	6.7 (1.2)
Total triglyceride (mmol/L)	1.7 (0.7)	2.5 (1.3)
HDL cholesterol (mmol/L)	1.0 (0.2)	1.0 (0.3)

ACE = angiotensin converting enzyme, HDL = high density lipoprotein

Figures in parentheses are SD or % of the relevant group as appropriate. There were no significant differences between the 2 groups

TABLE 14. PLASMA GLUCOSE AND INSULIN RESPONSES TO A 75 G ORAL GLUCOSE TOLERANCE TEST

	NGT (n=72)	IGT (n=18)	p value
Fasting			
Glucose (mmol/L)	5.1 (5.0 to 5.2)	5.1 (4.6 to 5.5)	0.836
Insulin (mU/L)	7.2 (6.0 to 8.6)	11.6 (7.5 to 17.9)	0.044
Insulin/Glucose (mU/mmol)	1.4 (1.2 to 1.7)	2.3 (1.4 to 3.8)	0.063
30 minute			
Glucose (mmol/L)	7.9 (7.5 to 8.2)	8.8 (8.2 to 9.3)	0.006
Insulin (mU/L)	62.3 (54.6 to 71.1)	83.3 (60.5 to 114.8)	0.098
Insulin/Glucose (mU/mmol)	8.1 (7.1 to 9.1)	9.5 (6.9 to 13.2)	0.331
120 minute			
Glucose (mmol/L)	5.8 (5.5 to 6.1)	8.9 (8.3 to 9.4)	< 0.001
Insulin (mU/L)	55.5 (46.7 to 66.0)	118.3 (96.2 to 145.3)	< 0.001
Insulin/Glucose (mU/mmol)	9.9 (8.5 to 11.4)	13.4 (10.7 to 16.7)	0.023
AUC			
Glucose (min.mmol/L)	852 (822 to 882)	1080 (1022 to 1137)	< 0.001
Insulin (min.mU/L)	8071 (7155 to 9104)	11861 (8901 to 15806)	0.022
Insulin/Glucose (mU/mmol)	9.6 (8.6 to 10.6)	11.0 (8.2 to 14.8)	0.388

AUC = area under the curve calculated from samples at 0, 30, 60, 90 and 120 minutes, NGT = normal glucose tolerance, IGT = impaired glucose tolerance, Values are arithmetic mean (95% CI) for glucose and geometric mean (95% CI) for insulin and insulin/glucose ratio, p value from Student's *t*-test for unpaired observations

TABLE 15. PEARSON CORRELATION COEFFICIENTS BETWEEN FASTING GLUCOSE 120 MINUTE GLUCOSE, FASTING INSULIN, 120 MINUTE INSULIN, BODY MASS INDEX, AND LEFT VENTRICULAR EJECTION FRACTION

	120 min glucose	Fasting insulin	120 min insulin	Body mass index	LVEF
Fasting glucose	0.106	-0.094	0.032	-0.026	0.049
120 min glucose	-	0.115	0.579***	0.117	0.042
Fasting insulin	-	-	0.42***	0.522***	-0.392**
120 min insulin	-	-	-	0.270*	-0.089
Body mass index	-	-	-	-	-0.21

LVEF = left ventricular ejection fraction

Correlation coefficients were calculated using log transformed data for fasting and 120 min insulin

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

TABLE 16. MULTIPLE LINEAR REGRESSION ANALYSIS FOR PREDICTORS OF FASTING INSULIN IN 56 PATIENTS WITH ISCHAEMIC HEART DISEASE

	Coefficient	Standard error	t-value	p value
Fasting glucose	-0.089	0.147	-.608	0.546
Body mass index	0.153	0.038	4.05	< 0.001
LVEF	-2.164	0.84	-2.575	0.013

LVEF = left ventricular ejection fraction

Calculations were performed using log transformed insulin data

TABLE 17. MULTIPLE LINEAR REGRESSION ANALYSIS FOR PREDICTORS OF 120 MINUTE INSULIN IN 56 PATIENTS WITH ISCHAEMIC HEART DISEASE

	Coefficient	Standard error	t-value	p value
Fasting glucose	0.009	0.121	0.074	0.942
Fasting insulin	0.312	0.114	2.751	0.008
120 minute glucose	0.239	0.048	5.03	< 0.001
Body mass index	0.008	0.035	0.234	0.816
LVEF	0.224	0.730	0.307	0.76

LVEF = left ventricular ejection fraction

Calculations were performed using log transformed insulin data

Figure 4. The glucose and insulin response to a 75 g oral glucose load in patients with ischaemic heart disease, 72 with normal glucose tolerance (NGT) and 18 with impaired glucose tolerance (IGT). Values are arithmetic mean (SD)

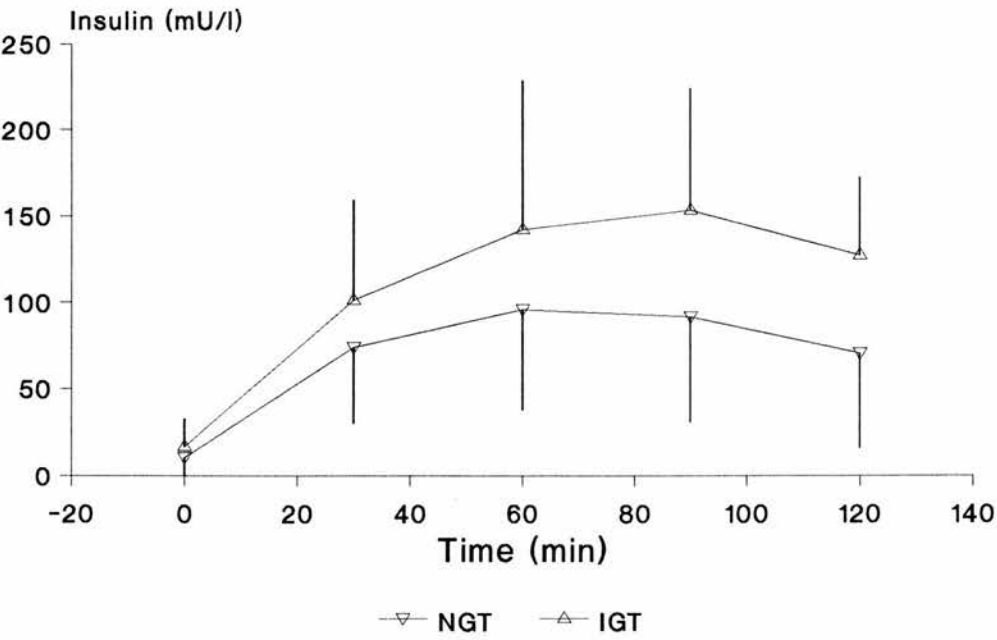
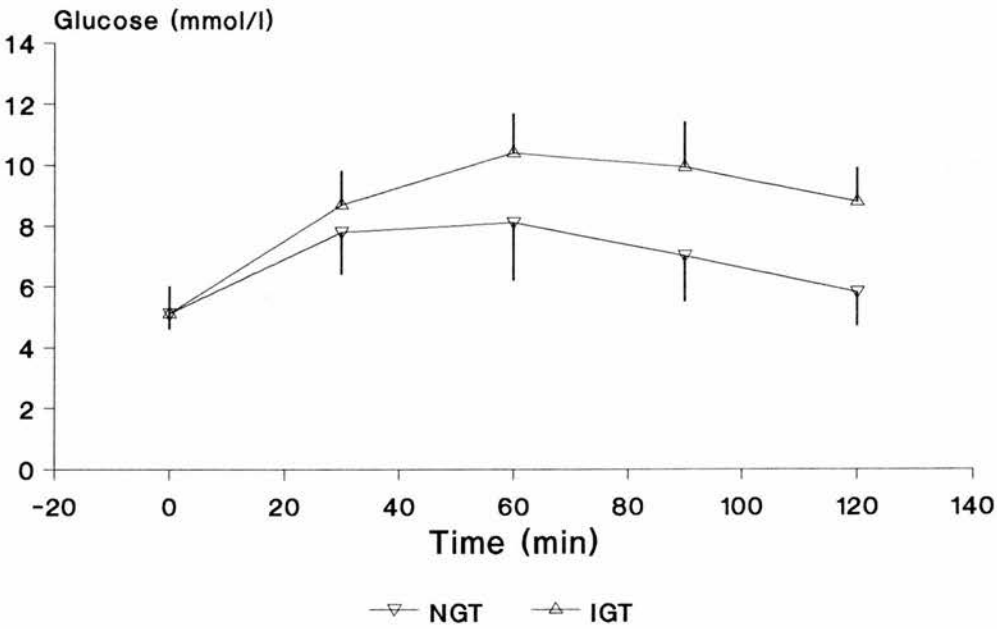


Figure 5. Plot of log fasting insulin against left ventricular ejection fraction (LVEF) with the regression line ($r = -0.392$, $p = 0.003$) and 95% CI of the regression line ($n = 56$). The equation for the regression line was $y = -2.9x + 3.03$. Patients with normal glucose tolerance are shown as open circles and those with impaired glucose tolerance as filled circles

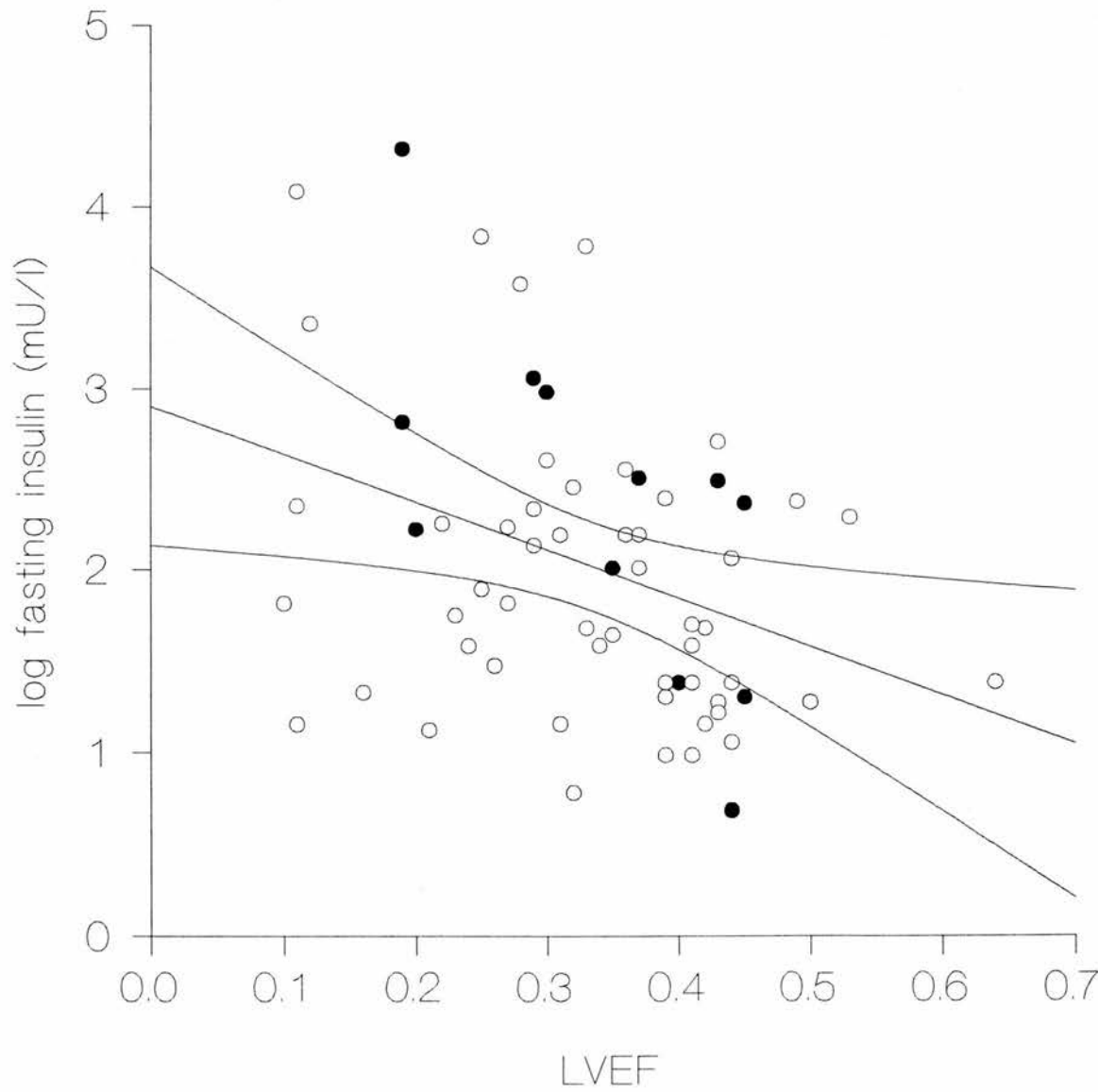
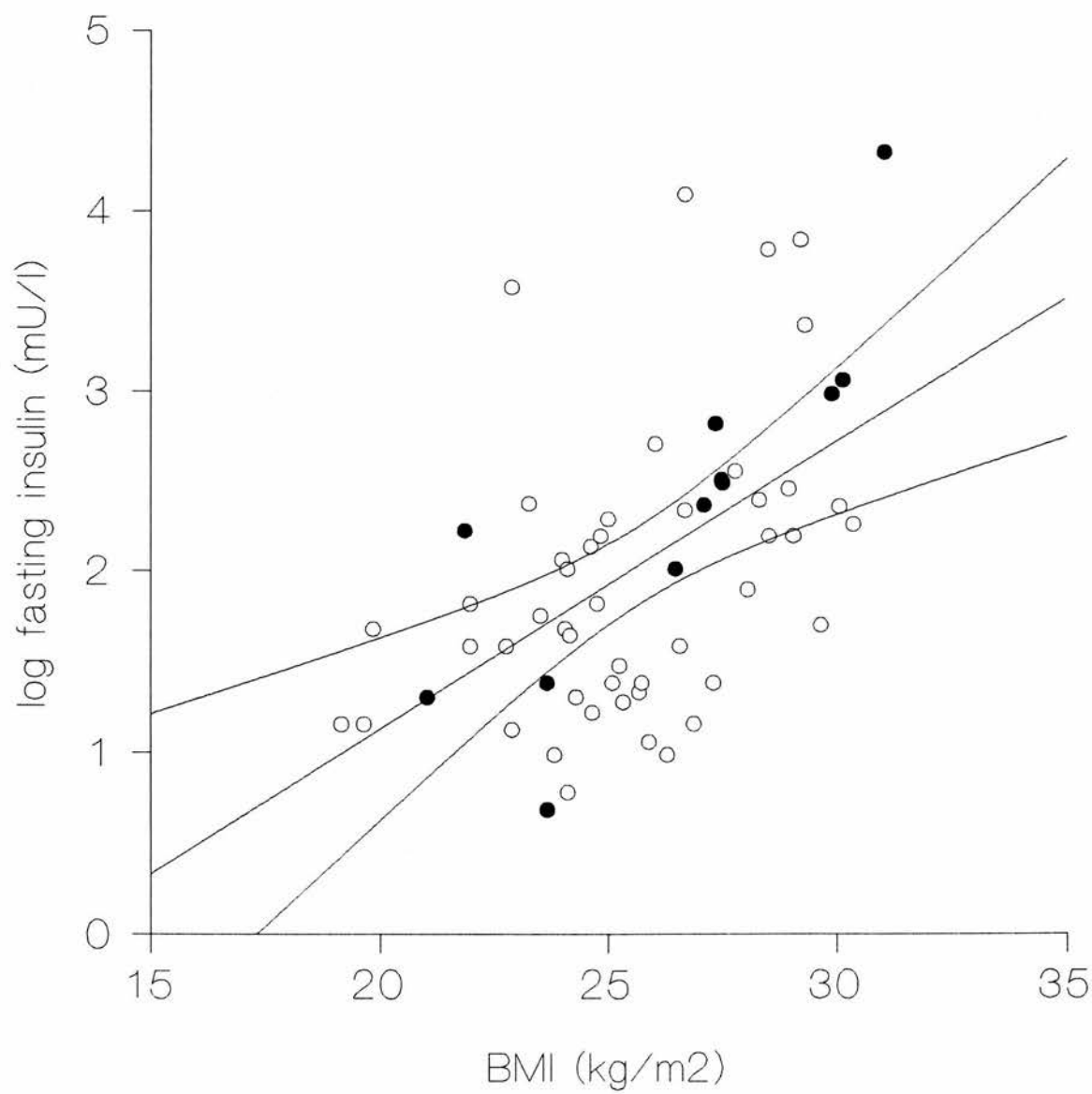


Figure 6. Plot of log fasting insulin against body mass index (BMI) with the regression line ($r = 0.522$, $p < 0.001$) and 95% CI of the regression line ($n = 56$). The equation for the regression line was $y = 0.17x - 2.46$. Patients with normal glucose tolerance are shown as open circles and those with impaired glucose tolerance as filled circles



CHAPTER 5

EFFECTS OF CAPTOPRIL ON HYPERINSULINAEMIA AND INSULIN RESISTANCE IN MEN WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION

5.1 SUMMARY

Angiotensin converting enzyme inhibitors have been shown to improve prognosis in patients with chronic heart failure and in patients with recent myocardial infarction. Captopril has been shown to increase insulin sensitivity in patients with essential hypertension. The study reported in this chapter examined the effect of captopril upon hyperinsulinaemia and insulin resistance in patients who had made an uncomplicated recovery from a recent myocardial infarction. Patients were randomised to receive 4 weeks placebo and 4 weeks captopril in a double-blind crossover study design. Hyperinsulinaemia and insulin sensitivity were assessed at the end of each treatment period using a 3 hour oral glucose tolerance test and a 2 hour euglycaemic hyperinsulinaemic clamp performed 48 hours apart. The use of captopril had no significant effect upon fasting or stimulated hyperinsulinaemia, or insulin sensitivity. This suggests that there may be differences in the mechanisms of insulin resistance in essential hypertension and ischaemic heart disease.

5.2 INTRODUCTION

Captopril has been shown to improve insulin sensitivity in patients with essential hypertension (Pollare et al 1989b) but, as discussed in Chapter 1, there have subsequently been conflicting reports for both captopril and other angiotensin converting enzyme (ACE) inhibitors which have been studied in subjects with essential hypertension (Santoro et al 1992; Egan 1993; Baba 1993; Uehara 1994; Oksa 1994), or diabetes mellitus (Seefeldt 1990; Uehara 1994), or both (Torlone 1991; Seghieri 1992; Bak 1992; Torlone 1993; Uehara 1994). The effect of ACE inhibitors upon insulin resistance has not been studied in patients with ischaemic heart disease.

Several very large studies of patients with acute myocardial infarction have suggested that the use of ACE inhibitors is associated with improved prognosis either in an unrestricted short term protocol (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico 1994), or over long periods in patients with either depressed left ventricular function assessed by radionuclide ventriculography (Pfeffer et al 1992), or with clinical evidence of heart failure (The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators 1993). The most likely mechanisms by which ACE inhibitors bring about their benefit is by an attenuation of left ventricular remodelling (Sharpe et al 1988; Pfeffer et al 1988), possibly by modulating the neurohumoral response to acute myocardial infarction (Rouleau et al 1993; Ray et al 1993).

The purpose of the studies presented in this and the next chapter was to investigate the effect of captopril on insulin resistance (this chapter) and endogenous fibrinolysis (Chapter 6) in a group of men who had recovered from an acute myocardial infarction without developing any complications such as heart failure or significant arrhythmia. To minimise the possible interference of other drug therapy, but yet to still investigate a group representative of a large number of patients with myocardial infarction, I elected to include only patients who were taking a cardioselective β adrenoceptor antagonist and aspirin, without other therapy. Thus I enrolled a group of patients who, at the time of study, did not have any conventional indications to undergo ACE inhibitor therapy and who were also taking those agents which had been shown to improve prognosis after myocardial infarction, aspirin (The ISIS-2 Collaborative Group 1988) and β blockade (The Norwegian Multicenter Group 1981; The Beta Blocker Heart Attack Research Group 1982).

5.3 METHODS

Subjects

Eighteen men younger than 75 years and suffering from their first myocardial infarction were recruited 6 weeks after their admission to the Coronary Care Unit at the Royal Infirmary of Edinburgh. In addition to a typical history, subjects had to have developed pathological Q waves on the 12 lead electrocardiogram or to have had a peak creatine kinase of > 800 iu/L, or both. All patients had been taking a cardioselective β adrenoceptor blocking agent and aspirin 300 mg daily from the time of their admission to hospital, and no other medication was allowed. None of

the patients who entered the study had previously taken an ACE inhibitor. Patients with a history of hypertension or diabetes mellitus, or with a family history of diabetes mellitus, were excluded because of the known association of these conditions with hyperinsulinaemia and insulin resistance. At the time of recruitment a full clinical assessment was made and a chest radiograph, symptom limited treadmill exercise test and radionuclide ventriculogram performed. Any patient with clinical or radiological signs of heart failure, or evidence of ischaemia or arrhythmia requiring additional therapy was excluded. Thus, the study population consisted of a group considered to have made an entirely uneventful recovery from their myocardial infarction and without any other significant illness.

Of the 18 patients who were initially entered into the study, 3 patients were subsequently excluded from analysis, one patient whose compliance with study medication was less than 90% by tablet count (75%), one patient whose β blocker was withdrawn by his general practitioner during the study because of mild dyspnoea, and one patient who moved to England during the study and was unwilling to continue to take part. Details of the index hospital admission for the 15 remaining patients are given in Table 18.

To provide data on values for insulin sensitivity in an age and body mass index matched healthy population, 12 men were identified at random from the Lothian Health Board register. None of these men gave a history of ischaemic heart disease, hypertension or diabetes mellitus, and none were taking any medication. None had a family history of diabetes mellitus. All of these healthy subjects had a normal physical examination and a normal 12 lead electrocardiogram.

All of the subjects who took part in this study gave fully informed oral and written consent to participate. In addition, their general practitioners were approached to ensure that they knew of no reason why their patients should not take part. The study was approved by the Lothian Health Board Ethical Committee.

Study design

The study was designed as a randomised, double-blind, placebo-controlled, crossover study. Entry into the study was 8 weeks after the onset of myocardial infarction in order to avoid any acute phase response or metabolic derangement around the time of infarction. A stable insulin response between weeks 3 and 12 after myocardial infarction is suggested by the data on oral glucose tolerance tests presented in Chapter 3.

At the beginning of each treatment period, patients received a test dose of placebo or 6.25 mg captopril under medical supervision and this was followed by one tablet of placebo or 25 mg captopril three times daily. After 4 weeks of therapy the patients crossed over to the other treatment arm for a further 4 weeks. No patient suffered a significant hypotensive response.

At the end of each treatment period, but separated by 48 hours, patients underwent an oral glucose tolerance test and a euglycaemic hyperinsulinaemic clamp study, thus each investigation was made at 12 and 16 weeks from the time of infarction. The healthy normal men underwent a euglycaemic clamp on one occasion only. Before each of these investigations subjects were asked to fast from 22.00 hours the previous night, but patients were asked to take their β blocker,

aspirin and captopril/placebo with a glass of water at 07.00 hours prior to attending the out patient department at 08.30 hours.

Oral glucose tolerance test

The patients were seated and a 16 G intravenous cannula was inserted into a large antecubital vein after which they rested for 30 minutes. Two baseline blood samples were taken 15 minutes apart before the patients took 75 g glucose monohydrate in 200 mL water. Further blood samples were taken at 30, 60, 90, 120, 150 and 180 minutes in an extended version of the standard test. Samples taken into fluoride oxalate for plasma glucose measurement were stored at room temperature and plasma separated within 1 hour prior to analysis within 3 hours. Glucose was measured using a Cobas Bio analyser by the hexokinase method (Schmidt 1973) with an interassay coefficient of variation of 1.7%. Samples taken into lithium heparin for immunoreactive insulin measurement were placed on ice and plasma was separated within 1 hour after centrifugation (20 minutes at 2000 g and 4°C). Plasma samples were stored at -40°C before analyses for the whole study were performed in Newcastle. Immunoreactive insulin was measured by radioimmunoassay (Soeldner and Slone 1965) with a coefficient of variation of 6.8-7.5%. Further details of this assay have been discussed in Chapter 2.

Euglycaemic hyperinsulinaemic clamp

The patients lay recumbent and two intravenous cannulae were inserted after which they rested for 30 minutes. An 18 G cannula was inserted into a large antecubital vein for infusion of insulin and 20% dextrose. A 21 G cannula was inserted retrogradely into a vein on the dorsum of the left hand and connected to a

continuous flush system to ensure patency. The left hand was placed inside a plexiglass heated water jacket maintained at an internal temperature of 55-65°C throughout the study to facilitate sampling of “arterialised” venous blood as discussed in Chapter 2. After 2 baseline “arterialised” blood samples taken 15 minutes apart, the euglycaemic hyperinsulinaemic clamp was performed according to the method of De Fronzo (De Fronzo et al 1979; see Discussion in Chapter 2). Essentially this consists of a 10 minute priming infusion of insulin followed by a continuous infusion of 40 mU/m² body surface area min, whilst after 4 minutes a variable infusion of 20% glucose solution (with added potassium chloride) is commenced and the rate varied in order to maintain the whole blood glucose concentration at the mean of the 2 baseline fasting samples. Measurements of whole blood glucose are made every 5 minutes using a glucose analyser (Yellow Springs Instruments, Yellow Springs, Ohio). Adjustments to the rate of infusion were guided according to the algorithm described by De Fronzo. The clamp was performed over at least 120 minutes and the aim was to achieve steady state blood glucose levels within 0.1 mmol/L of the target for the last 60 minutes. Additional blood samples were taken at 10 minute intervals over the last 60 minutes for measurement of plasma insulin by radioimmunoassay. Samples for insulin assay were handled exactly as described above. The measurement of insulin sensitivity was taken as the quotient of the whole body glucose intake (disposal) over the last 60 minutes and the prevailing plasma insulin concentration. This is a more true reflection of insulin sensitivity than merely the glucose disposal figure with no correction for the prevailing insulin concentration, although many investigators do only quote glucose disposal.

At the end of the study, the insulin infusion was stopped whilst the 20% glucose solution was continued for 30 minutes to prevent rebound hypoglycaemia. The plasma potassium was checked to see that it remained within normal range. Subjects received their lunch prior to leaving the hospital.

Statistical analysis

Descriptive data are given as mean (SEM). Incremental areas under the curve were calculated using the trapezium rule. Glucose data are given as arithmetic means (95% CI of the mean). Values for insulin data and insulin/glucose ratios were compared after logarithmic transformation to normalise their distribution and are presented as geometric mean (95% CI of the mean). The effect of captopril versus placebo was analysed by Student's *t* test for paired observations. Where comparisons are made between patients and healthy men, Student's *t* test for unpaired observations was used. Statistical analyses were made using SYSTAT for Windows, Version 5 (Systat Inc. 1992, Evanston, Illinois). The level of significance was $p < 0.05$.

5.4 RESULTS

Baseline characteristics

There were no significant differences in the baseline characteristics of the 12 normal men and the 15 patients (Table 19).

Captopril v placebo - oral glucose tolerance test

Nine patients received placebo first and 6 received captopril first in the crossover design. There was no evidence of an order effect and therefore results are

presented combined. The main results are given in Table 20 and the glucose and insulin curves for each treatment period are shown in Figure 7. Captopril had no effect upon glucose tolerance or fasting or stimulated hyperinsulinaemia.

Captopril v placebo - hyperinsulinaemic euglycaemic clamp

The main results are given in Table 21 and the plots of whole blood glucose, glucose infusion rate, and the plasma insulin concentration over the 120 minute period of the study are shown in Figure 8. The plot of blood glucose in Figure 8 confirms that identical blood glucose levels were maintained during the last 60 minutes of the two clamp studies. There was a significant decrease in whole body glucose disposal after 4 weeks treatment with captopril (323 v 299 mg/hr.kg, $p = 0.016$) but also a tendency for the mean plasma insulin concentration over the last 60 minutes to be lower (95.1 v 89.6 mU/L, $p = 0.124$). When the insulin sensitivity index was calculated, correcting whole body glucose disposal for the prevailing plasma insulin concentration, there was no difference between captopril and placebo effects (3.56 v 3.58 mg/hr.kg.mU/L plasma insulin, $p = 0.889$).

Captopril v placebo - other variables

The effect of 4 weeks therapy with captopril and placebo on blood pressure, plasma electrolytes and lipids is shown in Table 22. There were no significant differences.

Insulin sensitivity in patients v healthy normal subjects

The results for the hyperinsulinaemic euglycaemic clamp study in the 12 healthy normal men are given with those of patients taking a β blocker and aspirin with placebo only in Table 23. These are mainly intended to provide an indication of

the normal range of insulin sensitivity in age and body mass index matched controls. This group are not true controls for the patients because they were not taking aspirin or a β blocker. Taking heed of this caveat, statistical comparison between the healthy men and patients at the end of the placebo period revealed no significant difference in mean whole body glucose disposal ($360 \text{ v } 323 \text{ mg/hr.kg}$, $p = 0.439$) but with significantly lower prevailing plasma insulin in healthy subjects ($76.7 \text{ v } 95.1 \text{ mU/L}$, $p = 0.002$), and therefore a tendency to a higher insulin sensitivity index in healthy subjects ($4.89 \text{ v } 3.56 \text{ mg/hr.kg.mU/L plasma insulin}$, $p = 0.094$).

5.5 DISCUSSION

This study, performed on patients with previous uncomplicated myocardial infarction, has shown no evidence to support the hypothesis that captopril might improve hyperinsulinaemia and/or insulin resistance. The results of the oral glucose tolerance tests showed no influence of captopril on glucose tolerance or fasting insulin concentrations or the stimulated insulin response to an oral glucose load up to 180 minutes. The results of the euglycaemic hyperinsulinaemic clamp studies suggested that captopril might even have decreased whole body glucose disposal, although when this was corrected for the prevailing plasma insulin concentration to provide the insulin sensitivity index, there was no overall effect of captopril on insulin sensitivity. The similar plateau insulin level in response to a constant dose of insulin during the clamp and the identical insulin response to oral glucose also

suggest that neither the pattern of insulin clearance nor the secretory response of the pancreas were altered by captopril.

A number of studies have investigated the possible effect of ACE inhibitors on hyperinsulinaemia and insulin resistance in other patient groups and these were discussed in Chapter 1 and detailed in Table 3, Chapter 1. Including only studies in which captopril was given for more than 2 days, then there are three which merit consideration. In 24 patients with essential hypertension, after 4 months treatment with captopril 100 mg daily, there was a reduction in the stimulated insulin response to intravenous glucose and an increase in insulin sensitivity assessed by the euglycaemic clamp compared to baseline values (Pollare et al 1989b). In contrast, 14 hypertensive non-insulin dependent diabetic patients treated for 3 months with captopril 50-100 mg daily, showed no change in their insulin response to oral glucose or glucose disposal assessed by the insulin suppression test compared to baseline (Seghieri et al 1992). In neither of these studies was there a placebo control group. A single-blind, placebo controlled crossover study was performed in 16 hypertensive non-insulin dependent diabetic subjects who received captopril 50-100 mg daily or placebo for 3 months (Torlone et al 1993). Although a glucose tolerance test was not performed in this study, there was a significant fall in glycosylated haemoglobin, and euglycaemic clamp studies revealed improved insulin sensitivity, although there was no change in fasting insulin concentrations.

One problem in trying to compare with these other studies is that the nature of insulin resistance may differ in hypertension, ischaemic heart disease and non-insulin dependent diabetes; and thus the influence of ACE inhibition may not be the same

(reviewed by Donnelly 1992). In addition, an important difference between my study and the others cited is that the patients in my study maintained concomitant treatment with a β blocker and aspirin.

Although cardioselective β blockers are not thought to have a significant influence on glucose-insulin homeostasis (Sawicki and Berger 1992), they have been shown to influence insulin sensitivity in some studies (Pollare et al 1989a) and I cannot exclude the possibility that β_1 adrenoceptor antagonism obscured an effect of captopril in this study. A more intriguing possibility is that the use of aspirin may alter the response to ACE inhibition. This has recently been suggested with regard to the vasodilator effects of enalapril in heart failure (Hall et al 1992) although I am not aware of data regarding the effect of aspirin on the neurohumoral response to ACE inhibition.

Limitations of this study

It is possible that the duration of treatment in this study was too short, although some workers have reported an effect of ACE inhibition after only a few days or less (Torlone et al 1991; Gans et al 1991c; Uehara et al 1994). Acute myocardial infarction is known to be associated with a metabolic disturbance (Vetter et al 1974) and this might still influence glucose and insulin responses in the convalescent period. Against this hypothesis is the lack of a difference in hyperinsulinaemic response between patients with myocardial infarction studied at 3 weeks and 3 months presented in Chapter 3, and from this study, the lack of an order effect on glucose, insulin and lipid parameters within the crossover design. It is unlikely that there was any hangover effect for patients receiving captopril in the first

treatment period because of the described endocrine response to the withdrawal of captopril (Maslowski et al 1981), and specifically the findings of Pollare on the lack of a hangover effect of captopril on insulin sensitivity (Pollare et al 1989b). I purposely chose to study patients who had made an uncomplicated recovery from myocardial infarction without symptomatic left ventricular impairment. This was likely to minimise the neurohumoral disturbance associated with myocardial infarction, but at the same time may have reduced the likelihood of captopril having a beneficial effect. This was a deliberate strategy, prompted by the desire to explore possible new indications for the use of ACE inhibitors in patients following myocardial infarction rather than investigating patients for whom there were already good indications for their use.

Insulin sensitivity in patients with myocardial infarction and healthy men

Although the results of the euglycaemic hyperinsulinaemic clamp in the healthy subjects were intended to provide a normal range, and they were not controls because they were not taking a β blocker or aspirin, a number of interesting observations can still be made. Firstly, although cardioselective β blockers have been shown to worsen insulin resistance in essential hypertension (Pollare et al 1989a), the patients were not significantly more insulin resistant than the healthy men, even though the trend was in that direction. Secondly, for a given dose of intravenous insulin (40 mU/m^2 body surface area min), the healthy men had a significantly lower plateau plasma insulin concentration. This suggests that insulin clearance is impaired, either as an effect of β blockade, which has been suggested before (Pollare et al 1989a), or due to impaired insulin clearance as a feature of ischaemic heart disease.

Impaired insulin clearance has been described in hypertension (Salvatore et al 1992).

Overall it is perhaps surprising that there was no difference in glucose disposal or insulin sensitivity index between the patients and healthy mean, although the trend was in that direction. This reflects the limited power of the study to detect a difference because of the large variance of the observation. In addition, the nature of the patients studied made them less likely to be insulin resistant because of the absence of heart failure. In a study of 8 patients with chronic congestive heart failure, of whom only one had ischaemic heart disease, the patients were shown to have impaired insulin sensitivity which correlated strongly with plasma concentrations of noradrenaline (Paolisso et al 1991). Two recent studies of insulin sensitivity amongst patients with ischaemic heart disease have shown insulin resistance using either the insulin suppression test (Young et al 1993) or an intravenous glucose tolerance test and mathematical model (Ley et al 1994). However, formal measurement of left ventricular function was not made in these studies, the subjects contained a mixture of those with and without previous myocardial infarction, and patients with impaired glucose tolerance did not appear to be excluded.

5.6 CONCLUSION

This study has shown no evidence to support the hypothesis that captopril might improve insulin resistance in patients with uncomplicated myocardial infarction taking a β blocker and aspirin.

TABLE 18. DETAILS OF THE INDEX MYOCARDIAL INFARCTION IN THE 15 PATIENTS

	n or mean (range)
Cigarette smokers	3
Family history of IHD	3
Site-anterior	8
Site-inferior	7
Q wave	14
Streptokinase therapy	14
Peak creatine kinase (iu/L)	1900 (476 - 4259)
Cardiothoracic ratio	0.48 (0.40 - 0.55)
LVEF at 6 weeks	0.37 (0.28 - 0.42)

IHD = ischaemic heart disease

LVEF = left ventricular ejection fraction

TABLE 19. BASELINE CHARACTERISTICS OF THE NORMAL MEN AND PATIENTS WITH RECENT MYOCARDIAL INFARCTION

	Normals (n=12)	Patients (n=15)
Age (years)	57 (2)	63 (2)
Height (m)	1.77 (0.02)	1.78 (0.01)
Weight (kg)	79.5 (2.2)	79.5 (2.2)
BMI (kg/m ²)	25.4 (0.6)	25.2 (0.6)
Pulse (bpm)	67 (2)	60 (2)
Systolic BP (mm Hg)	120 (4)	124 (5)
Diastolic BP (mm Hg)	80 (3)	77 (3)
Cholesterol (mmol/L)	6.2 (0.3)	6.9 (0.2)
Triglyceride (mmol/L)	1.5 (0.2)	2.0 (0.2)
HDL Cholesterol (mmol/L)	1.3 (0.1)	1.1 (0.1)
Glucose (mmol/L)	4.9 (0.1)	5.2 (0.1)
Insulin (mU/L)	7.3 (1.8)	7.7 (2.3)

BMI = body mass index, HDL = high density lipoprotein

Data are presented as mean (SEM)

TABLE 20. PLASMA GLUCOSE AND INSULIN RESPONSES TO A 75 G ORAL GLUCOSE TOLERANCE TEST IN 15 PATIENTS WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION AFTER 4 WEEKS TREATMENT WITH PLACEBO AND AFTER 4 WEEKS TREATMENT WITH CAPTOPRIL

	Placebo	Captopril	p value
Fasting			
Glucose (mmol/L)	5.2 (5.0 to 5.5)	5.2 (4.9 to 5.5)	0.736
Insulin (mU/L)	5.2 (3.2 to 8.2)	5.0 (3.3 to 7.8)	0.866
Insulin/Glucose (mU/mmol)	1.0 (0.6 to 1.5)	1.0 (0.6 to 1.5)	0.911
120 minute			
Glucose (mmol/L)	6.5 (5.6 to 7.5)	6.7 (5.5 to 7.9)	0.757
Insulin (mU/L)	53.6 (35.0 to 82.1)	58.9 (39.1 to 88.6)	0.633
Insulin/Glucose (mU/mmol)	8.4 (5.7 to 12.6)	9.3 (6.6 to 13.1)	0.515
AUC (180 min)			
Glucose (min.mmol/L)	235 (133 to 336)	265 (158 to 372)	0.453
Insulin (min.mU/L)	8553 (6266 to 11674)	8468 (6283 to 11411)	0.909
Insulin/Glucose (min.mU/mmol)	51.3 (25.6 to 103.0)	38.2 (23.8 to 61.1)	0.230

AUC = incremental area under the curve from 0 to 180 minutes

Results are arithmetic mean (95% CI) for glucose and geometric mean (95% CI) for insulin and insulin glucose ratios

Statistical testing was by Student's *t* test for paired observations using the raw data for glucose results and log transformed data for insulin and insulin/glucose ratios

TABLE 21. PRINCIPAL RESULTS OF THE HYPERINSULINAEMIC EUGLYCAEMIC CLAMP STUDIES IN 15 PATIENTS WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION AFTER 4 WEEKS TREATMENT WITH PLACEBO AND AFTER 4 WEEKS TREATMENT WITH CAPTOPRIL.

	Placebo	Captopril	p value
Rate of infusion of 20% glucose in the last hour⁽¹⁾ (mL/hr)	129 (104 to 154)	120 (95 to 144)	0.015
Whole body glucose disposal in the last hour⁽²⁾ (mg.hr ⁻¹ .kg ⁻¹)	323 (266 to 381)	299 (245 to 353)	0.016
Mean plasma insulin in the last hour (mU/L)	95.1 (86.4 to 103.8)	89.6 (80.3 to 98.9)	0.124
Insulin sensitivity index⁽³⁾ (mg.hr ⁻¹ .kg ⁻¹ .mU ⁻¹ .l)	3.56 (2.72 to 4.40)	3.58 (2.61 to 4.56)	0.889

Results are arithmetic mean (95% CI)

Statistical testing was by Student's *t* test for paired observations

Notes

- (1) Rate of 20% glucose infusion (mL/hr) is raw data not corrected for body weight.
- (2) Whole body glucose disposal in the last hour (mg.hr⁻¹.kg⁻¹) is corrected for body weight but not corrected for the prevailing plasma insulin concentration.
- (3) The insulin sensitivity index (mg.hr⁻¹.kg⁻¹.mU⁻¹.l) represents the whole body glucose disposal in the last hour corrected for body weight *and* the prevailing plasma insulin concentration.

TABLE 22. EFFECT OF 4 WEEKS PLACEBO AND 4 WEEKS CAPTOPRIL ON OTHER PARAMETERS

	Placebo	Captopril
Systolic BP (mm Hg)	117 (4)	114 (4)
Diastolic BP (mm Hg)	69 (3)	68 (4)
Urea (mmol/L)	6.3 (0.3)	6.5 (0.4)
Sodium (mmol/L)	141 (1)	141 (1)
Potassium (mmol/L)	4.1 (0.1)	4.1 (0.1)
Creatinine (μmol/L)	105 (3)	105 (3)
Cholesterol (mmol/L)	6.9 (0.2)	6.8 (0.2)
Triglyceride (mmol/L)	2.0 (0.2)	2.2 (0.3)
HDL cholesterol (mmol/L)	1.1 (0.1)	1.1 (0.1)

HDL = high density lipoprotein

Values are mean (SEM)

TABLE 23. PRINCIPAL RESULTS OF THE HYPERINSULINAEMIC EUGLYCAEMIC CLAMP STUDIES IN 12 HEALTHY MEN AND IN 15 PATIENTS WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION AFTER 4 WEEKS TREATMENT WITH PLACEBO

	Healthy Men	Patients	p value
Rate of infusion of 20% glucose in the last hour⁽¹⁾ (mL/hr)	140 (113 to 166)	129 (104 to 154)	0.535
Whole body glucose disposal in the last hour⁽²⁾ (mg.hr ⁻¹ .kg ⁻¹)	360 (278 to 442)	323 (266 to 381)	0.439
Mean plasma insulin in the last hour (mU/L)	76.7 (69.1 to 84.3)	95.1 (86.4 to 103.8)	0.002
Insulin sensitivity index⁽³⁾ (mg.hr ⁻¹ .kg ⁻¹ .mU ⁻¹ .l)	4.89 (3.52 to 6.26)	3.56 (2.72 to 4.40)	0.094

Results are arithmetic mean (95% CI)

Statistical testing was by Student's *t* test for unpaired observations

Notes

- (1) Rate of 20% glucose infusion (mL/hr) is raw data not corrected for body weight.
- (2) Whole body glucose disposal in the last hour (mg.hr⁻¹.kg⁻¹) is corrected for body weight but not corrected for the prevailing plasma insulin concentration.
- (3) The insulin sensitivity index (mg.hr⁻¹.kg⁻¹.mU⁻¹.l) represents the whole body glucose disposal in the last hour corrected for body weight *and* the prevailing plasma insulin concentration.

Figure 7. Plasma glucose and insulin response to a 75 g oral glucose tolerance test in 15 patients with recent uncomplicated myocardial infarction after 4 weeks placebo (×) and 4 weeks captopril (○). Values are mean (SD)

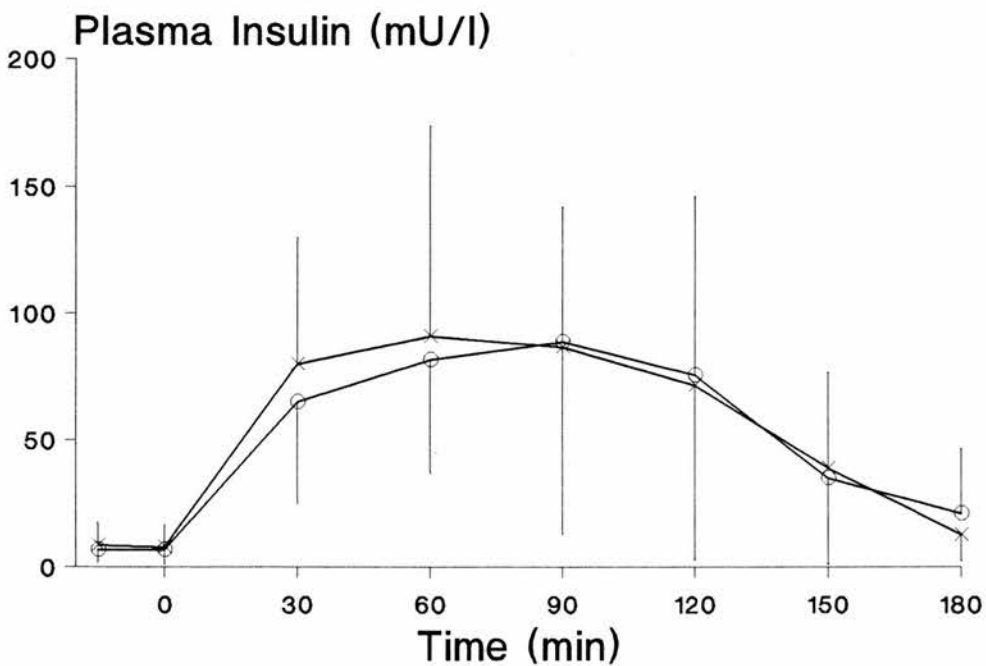
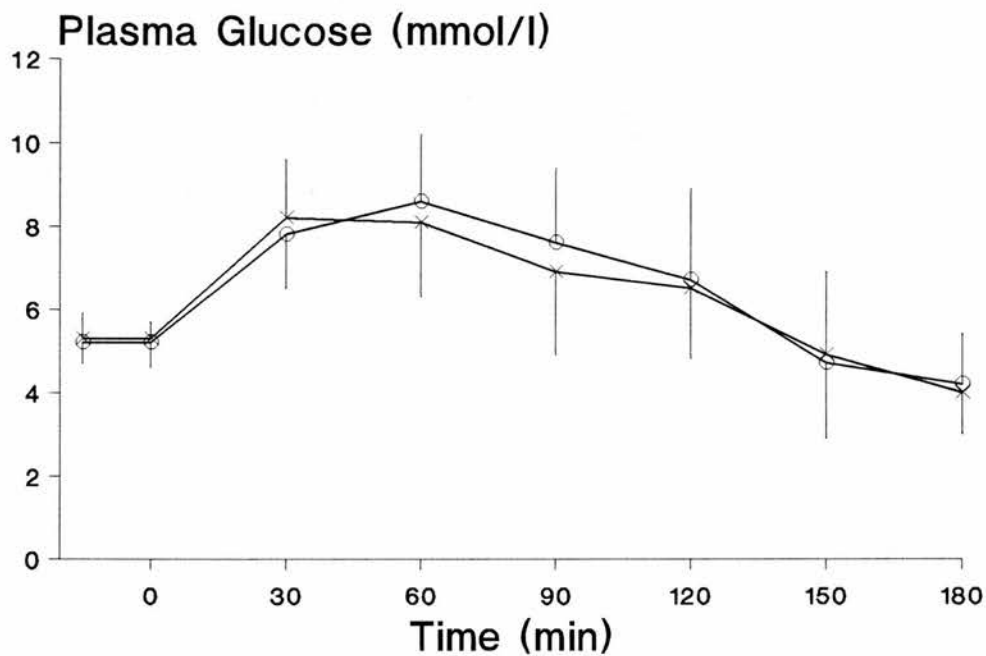
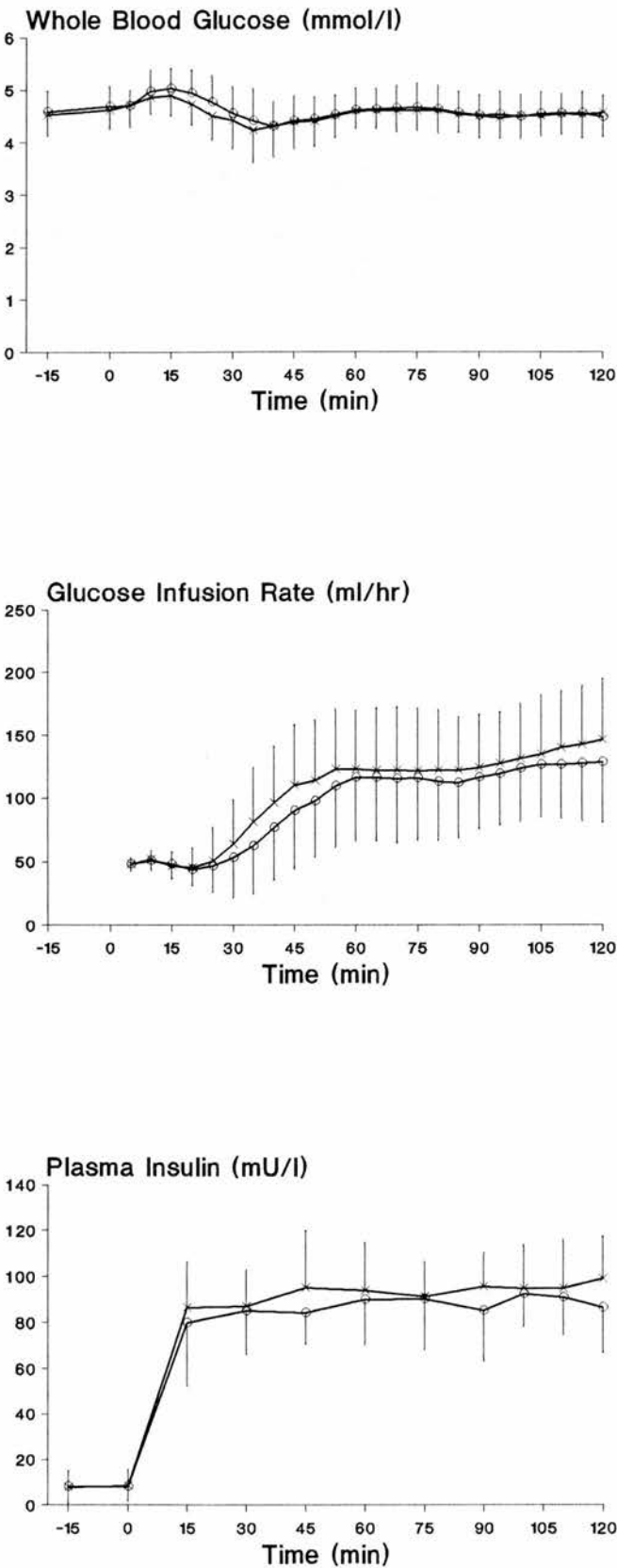


Figure 8. The whole blood glucose concentration, infusion rate of 20% glucose solution, and plasma insulin concentrations during a euglycaemic clamp performed in 15 patients with recent uncomplicated myocardial infarction after 4 weeks placebo (×) and after 4 weeks captopril (○). Values are mean (SD)



CHAPTER 6

EFFECTS OF CAPTOPRIL ON ENDOGENOUS FIBRINOLYSIS IN MEN WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION

6.1 SUMMARY

Increased plasma concentrations of tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor type 1 (PAI-1) activity have been associated with an adverse prognosis in patients with ischaemic heart disease. The use of angiotensin converting enzyme inhibitors has been shown to improve prognosis after myocardial infarction. The study in this chapter examined the effect of captopril on t-PA antigen and PAI-1 antigen and activity in patients who had made an uncomplicated recovery from recent myocardial infarction. Patients were randomised to receive 4 weeks placebo and 4 weeks captopril in a double-blind crossover design. Treatment with captopril was associated with a significant reduction in t-PA antigen and PAI-1 activity. This may help to explain the diminished risk of coronary thrombosis associated with the use of angiotensin converting enzyme inhibitors.

6.2 INTRODUCTION

Data from three large placebo controlled studies have indicated that the administration of angiotensin converting enzyme inhibitors to patients with mild left ventricular dysfunction following myocardial infarction results in a reduced incidence of acute coronary syndromes (The SOLVD Investigators 1991; The SOLVD Investigators 1992; Pfeffer et al 1992). The mechanism by which this is achieved is not clear, but suggestions have included effects upon blood pressure, angiotensin II, or the arterial intima (Yusuf et al 1992).

Impaired endogenous fibrinolysis is associated with an increased risk of intravascular thrombosis (Astrup 1956). The activity of the fibrinolytic system is reflected in circulating levels of tissue-type plasminogen activator and its most physiologically important inhibitor, plasminogen activator inhibitor type 1 (Kruithof et al 1988). Raised levels of tissue-type plasminogen activator antigen have been shown to be a marker of risk for coronary thrombosis in healthy men (Ridker et al 1993b), in patients with angina (Munkvad 1990, Jansson et al 1991) and in patients with myocardial infarction (Gram et al 1987b), and most recently to predict long term mortality in patients with coronary artery disease (Jansson et al 1993). Elevated levels of plasminogen activator inhibitor type 1 activity have been identified in young survivors of myocardial infarction (Hamsten 1985) and were also shown to be associated with an increased risk of reinfarction (Hamsten et al 1987b). Studies in normal subjects (Vague et al 1986) and patients with angina (Juhan-Vague et al 1989a) have shown a correlation between fasting insulin and both tissue-type

plasminogen activator and plasminogen activator inhibitor type 1. This is supported by in vitro evidence that insulin increases the production of plasminogen activator inhibitor type 1 by hepatocytes (Alessi et al 1988, Kooistra et al 1989). Angiotensin converting enzyme inhibitors have been shown to improve hyperinsulinaemia in hypertensive patients (Pollare et al 1989b), and thus might influence endogenous fibrinolysis via this pathway. Recently it has been shown that an intravenous infusion of angiotensin II results in an increase in plasma levels of plasminogen activator inhibitor type 1 (Ridker et al 1993a), providing a link between the renin-angiotensin system and risk of thrombosis, and an alternative path by which angiotensin converting enzyme inhibitors might influence fibrinolysis.

The hypothesis that the angiotensin converting enzyme inhibitor captopril might modify endogenous fibrinolysis was investigated in men following myocardial infarction which was uncomplicated by clinically manifest heart failure, arrhythmia or recurrent ischaemia.

6.3 METHODS

Subjects

Eighteen men younger than 75 years and suffering from their first myocardial infarction were recruited 6 weeks after their admission to hospital. In addition to a typical history, subjects had to show pathological Q waves in the ECG or have had a peak creatine kinase of > 800 iu/L, or both. Patients with a history of hypertension or diabetes mellitus were excluded. All patients had been taking a cardioselective β adrenoceptor blocking agent and 300 mg of aspirin daily from the time of

admission to hospital, and no other medication was permitted. None had previously taken an angiotensin converting enzyme inhibitor. At the time of recruitment a full clinical assessment was made and a chest radiograph, symptom limited treadmill exercise test and radionuclide ventriculogram performed. Any patient with clinical or radiological signs of heart failure, or evidence of ischaemia or arrhythmia requiring additional therapy was excluded, and only those considered to have made an uncomplicated recovery from their infarction and without other significant illness were admitted to the study. Three patients were excluded from analysis, one patient whose compliance was less than 90% by tablet count (75%), one patient whose β blocker was withdrawn by his general practitioner during the study and one who moved to England during the study and was unwilling to continue with study medication. Details of the index admission to hospital for the 15 patients are given in Table 18, Chapter 5.

To provide comparison for fibrinolytic parameters, 12 normal men of similar age to the patients were identified at random from the Lothian Health Board register. None of these men gave a history of ischaemic heart disease, hypertension or diabetes mellitus and none were taking any medication. They all had a normal physical examination and normal 12 lead ECG.

All subjects gave their oral and written consent to participate in the study and the study was approved by the Lothian Health Board Ethical Committee.

Study design

The study was designed as a randomised, double-blind, placebo-controlled crossover study. Entry into the study was 8 weeks after the onset of myocardial

infarction in order to avoid any acute phase response or short term fluctuation in fibrinolytic function (Gram et al 1987a). Patients received a test dose of placebo or 6.25 mg captopril under medical supervision at the beginning of each treatment period and this was followed by one tablet of placebo or 25 mg captopril three times daily. After 4 weeks of therapy the patients crossed over to the other treatment arm for a further 4 weeks. No patient suffered a significant hypotensive response. Measurements of fibrinolytic and other parameters were made on one occasion only for normal subjects and at the end of each 4 week treatment period for patients, thus at 12 and 16 weeks from the onset of myocardial infarction. Patients fasted from 22.00 hours the previous night, took their β blocker, aspirin and placebo/captopril with a glass of water at 07.00 hours and attended the out patient department at 08.30 hours. The patients lay recumbent, a 16 G intravenous cannula was inserted into a large antecubital vein and flushed with saline. Thirty minutes later 5 mL of blood was drawn and discarded, a further 10 mL of blood was taken into potassium citrate anticoagulant for fibrinolytic assays, 10 mL into lithium heparin for immunoreactive insulin, urea and electrolytes, 2.5 mL into fluoride oxalate for glucose and 10 mL into a plain glass tube for serum lipoproteins. Ten minutes later a further 5 mL of blood was drawn and discarded, and an additional 10 mL sample for fibrinolytic assays was taken into potassium citrate. The samples for fibrinolytic assays and insulin were placed into melting ice. Within 1 hour, plasma was separated by centrifugation (20 min at 2000 g and -4°C), immediately frozen in dry ice and stored at -40°C .

Assays

All fibrinolytic assays were performed at the same time after completion of the study. Assays for tissue-type plasminogen activator and plasminogen activator inhibitor type 1 antigen were by a two-site enzyme-linked immunosorbent assay (Biopool AB, Umea, Sweden) and were performed as instructed by the manufacturer. This follows the method of Ranby (Ranby et al 1986) in which plasma samples are incubated in microtitre plates coated with monoclonal antibodies against the relevant antigen, unbound antigens are washed off and bound antigen detected by addition of a second specific antibody conjugated to horseradish peroxidase. Standard curves were constructed using purified antigen diluted in plasma to known concentrations. The amount of tissue-type plasminogen activator and plasminogen activator inhibitor type 1 antigen in samples was deduced by comparing absorbence with the standard curve. The coefficients of variation for repeated measures of tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 antigen in our laboratory were 9.2% and 5.5% respectively. Plasminogen activator inhibitor type 1 activity was measured by chromogenic assay (Biopool AB, Umea, Sweden) according to the manufacturer's instructions and according to the principle first presented by Chmielewska (Chmielewska et al 1983). Briefly, a fixed amount of tissue-type plasminogen activator is added to the plasma sample and allowed to react with the plasminogen activator inhibitor type 1 present. The residual tissue-type plasminogen activator activity is measured by its ability to catalyse the conversion of plasminogen to plasmin assessed colorimetrically and compared to standard curves. The plasminogen activator inhibitor type 1 activity is defined as the difference between

the amount of tissue-type plasminogen activator added and the amount of tissue-type plasminogen activator found. The coefficient of variation for repeated measures of plasminogen activator inhibitor type 1 activity in our laboratory was 5.9%. Total immunoreactive insulin was measured by radioimmunoassay (Soeldner and Slone 1965). Urea and electrolytes were measured on an autoanalyser. Total plasma cholesterol and triglycerides were determined enzymatically and HDL cholesterol using magnesium dextran. Plasma glucose was measured using the hexokinase method (Cobas Bio analyser).

Statistical analyses

For fibrinolytic parameters the mean of 2 samples taken 10 minutes apart was calculated and the values are presented as median (range) because of their skewed distribution. Other parameters are given as mean (SE). Comparison of fibrinolytic parameters between the normal subjects and myocardial infarction patients taking placebo was by Mann Whitney U test. Comparison of fibrinolytic parameters between the myocardial infarction patients at the end of each treatment period was by Wilcoxon rank sum test and the estimated median and 95% CI were calculated using Minitab Release 7 (Minitab Inc. 1989, Pennsylvania) on a personal computer. Comparison of other parameters between the groups was by paired or unpaired Student's *t* test as appropriate. To examine relationships between haemostatic variables and metabolic parameters for all subjects (normals and myocardial infarction patients on placebo) a Pearson correlation matrix using log transformed data for variables which were not normally distributed (insulin, tissue-type plasminogen activator, plasminogen activator inhibitor type 1 antigen, plasminogen

activator inhibitor type 1 activity) was constructed with SYSTAT for Windows Version 5 (Systat Inc. 1992, Evanston, Illinois) on a personal computer. The significance level for correlations is given after applying the Bonferroni correction for multiple comparisons. All reported p values are two-tailed.

6.4 RESULTS

Baseline characteristics (Table 19, Chapter 5)

There were no significant differences in the baseline characteristics of the 12 normal men and the 15 patients.

Fibrinolytic parameters (Results are summarised in Table 24)

Normal Men v Myocardial Infarction Patients

Tissue-type plasminogen activator antigen levels at the end of the placebo period were significantly elevated in patients compared to normal men ($p = 0.001$). Plasminogen activator inhibitor type 1 antigen tended to be higher in patients and plasminogen activator inhibitor type 1 activity was significantly increased ($p = 0.04$).

Myocardial Infarction Patients - Placebo v Captopril

Nine patients received placebo first and 6 received captopril first in the crossover design. There was no evidence of an order effect and therefore summary results are presented combined in Table 24, individual results are given in the figures.

Tissue-Type Plasminogen Activator Antigen (Figure 9)

After 4 weeks therapy with captopril compared to 4 weeks placebo, there was a highly significant fall in tissue-type plasminogen activator ($p = 0.001$) with a

median reduction of 46% (95% CI, -29 to -64%). Fourteen patients showed a reduction and one showed a small rise.

Plasminogen Activator Inhibitor Type 1 Antigen (Figure 10)

After captopril compared to placebo, 10 patients showed a fall in plasminogen activator inhibitor type 1 antigen, one patient no change, and 4 patients showed a rise. There was no overall significant effect, although there was a median reduction of 18% (+9 to -49%, $p = 0.17$).

Plasminogen Activator Inhibitor Type 1 Activity (Figure 11)

After captopril there was a significant reduction in plasminogen activator inhibitor type 1 activity ($p = 0.02$) with a median reduction of 17% (-8 to -29%), 13 patients showed a reduction in plasminogen activator inhibitor type 1 activity and 2 showed a rise.

Other parameters (Table 22, Chapter 5)

Myocardial Infarction Patients - Placebo v Captopril

After 4 weeks therapy with placebo compared to 4 weeks captopril, there was no significant effect upon blood pressure or plasma urea, electrolytes, lipoproteins, glucose and insulin.

Relationship between metabolic and haemostatic parameters for all subjects

Using combined results from normal men and patients with myocardial infarction treated with β blocker, aspirin and placebo, Table 25 shows the Pearson correlation coefficients between fibrinolytic parameters and plasma glucose, insulin

and lipids. After adjustment for multiple comparisons, the correlations between tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 activity ($r = 0.61$, $p = 0.027$), and between plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor type 1 activity ($r = 0.673$, $p = 0.005$), remained significant. Plasminogen activator inhibitor type 1 activity also showed significant correlations with fasting insulin ($r = 0.591$, $p = 0.041$) and with fasting triglyceride ($r = 0.596$, $p = 0.037$).

6.5 DISCUSSION

Episodes of unstable angina or further infarction have a considerable influence on prognosis after a first myocardial infarction. The balance between thrombosis and fibrinolysis is central to the evolution of these acute coronary syndromes. The results presented in this chapter have shown that there is abnormal activation of the endogenous fibrinolytic system as shown by elevated levels of tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 activity in male survivors of uncomplicated acute myocardial infarction. In addition, 4 weeks treatment with captopril (75 mg daily) in a double-blind, placebo-controlled, randomised crossover study was associated with a significant fall in tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 activity. Angiotensin converting enzyme inhibitors have not previously been reported to have an effect on fibrinolytic parameters and this may help to explain why their use is associated with a reduction in risk of acute coronary thrombosis.

Endogenous fibrinolysis in ischaemic heart disease

Elevated levels of tissue-type plasminogen activator antigen at baseline were associated with a greater risk of subsequent myocardial infarction in the Physicians' Health Study (Ridker et al 1993b). Two studies have shown that the risk of subsequent cardiovascular events in patients with unstable angina was predicted by tissue-type plasminogen activator antigen but not by plasminogen activator inhibitor type 1 activity (Munkvad 1990b; Jansson et al 1991), and in a study of patients with prior myocardial infarction those who suffered reinfarction had higher tissue-type plasminogen activator antigen but lower tissue-type plasminogen activator activity (Gram et al 1987a and 1987b). A recent study in 213 patients with coronary artery disease has shown that concentrations of tissue-type plasminogen activator antigen predict mortality over a 7 year follow up (Jansson et al 1993). An earlier study of young survivors of myocardial infarction (Hamsten 1985) also found higher levels of tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 activity than controls, and a greater risk of further infarction was predicted by increased levels of plasminogen activator inhibitor type 1 activity in a study in which the results for tissue-type plasminogen activator antigen were not reported (Hamsten et al 1987b). Thus, it has been a consistent finding that both tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor type 1 activity are elevated in patients with ischaemic heart disease, whereas tissue-type plasminogen activator activity is decreased. It should be noted that the results of assays which measure levels of antigen include inactive complexes of tissue-type plasminogen activator with plasminogen activator inhibitor

type 1, and therefore it is valuable to have measures of both antigen and activity. It might initially be thought that elevated levels of tissue-type plasminogen activator antigen would suggest a decreased risk of thrombosis by indicating a more active fibrinolytic system. One hypothesis which could help to explain these observations is that increased secretion of these molecules, measured by the levels of antigen detected, reflect a response to stimuli which promote thrombosis, and that it is these which increase the risk of subsequent thrombotic occlusive events. This hypothesis is supported by evidence that both thrombosis and pharmacological thrombolysis increase circulating levels of plasminogen activator inhibitor type 1 (Fujii et al 1991) and the expression of the plasminogen activator inhibitor type 1 gene within the endothelium (Fujii et al 1992). It is likely that increased circulating plasminogen activator inhibitor type 1 stimulates release of tissue-type plasminogen activator, which is supported by the strong correlation between tissue-type plasminogen activator antigen and plasminogen activator inhibitor type 1 activity seen in this study and reported by others (Olofsson et al 1989). Acute variations in endogenous fibrinolytic activity may be best assessed by plasminogen activator inhibitor type 1 activity, since this is the predominant physiological governor of tissue-type plasminogen activator action. Plasminogen activator inhibitor type 1 exhibits marked diurnal fluctuation (Angleton et al 1989), whereas tissue-type plasminogen activator has much less diurnal change, which may have implications for clinical studies and help to explain why levels of tissue-type plasminogen activator antigen predict risk of future clinical events (Ridker et al 1993b; Munkvad 1990b; Jansson et al 1991;

Gram et al 1987a and 1987b) and results for plasminogen activator inhibitor type 1 are less consistent (Munkvad 1990b; Jansson et al 1991; Hamsten et al 1987b).

Possible mechanisms in this study

In this study, the influence of captopril on fibrinolytic function occurred despite there being no effect upon plasma insulin, glucose, electrolytes, and lipoproteins. It is important to note, however, that as previously reported (Vague et al 1986; Juhan-Vague et al 1989), there was a positive correlation between plasminogen activator inhibitor type 1 activity and both fasting insulin and triglyceride, although each accounts for less than 40% of the variability of plasminogen activator inhibitor type 1 activity. It may be that the lack of a more marked effect upon plasminogen activator inhibitor type 1 in this study reflects the absence of a change in metabolic variables with captopril, but at the same time our results indicate that factors other than these must be important in the regulation of both plasminogen activator inhibitor type 1 and tissue-type plasminogen activator. The small fall in blood pressure with captopril, which was not significant in this study, was comparable to that reported in the SAVE and SOLVD studies (The SOLVD Investigators 1991; The SOLVD Investigators 1992; Pfeffer et al 1992). Although angiotensin converting enzyme inhibitors have been shown to reduce insulin levels in hypertensive subjects (Pollare et al 1989b), this effect has not been found in all groups (Seghieri et al 1992 and discussed in Chapters 1 and 5). Recently it has been suggested that the renin-angiotensin system may play a more direct role in the control of endogenous fibrinolysis (Ridker et al 1993a). In a study in hypertensive subjects, an intravenous infusion of angiotensin II caused a significant rise in plasminogen activator inhibitor

type 1 antigen with no effect upon tissue-type plasminogen activator antigen (Ridker et al 1993a). As angiotensin converting enzyme inhibitors inhibit the formation of angiotensin II, they might be expected to cause a fall in levels of plasminogen activator inhibitor type 1, but this would not explain the decrease in tissue-type plasminogen activator. Angiotensin converting enzyme inhibitors also influence the kinin system and lead to increased levels of bradykinin (Johnston et al 1982). Tissue-type plasminogen activator is primarily secreted from the vascular endothelium, from which its acute release is enhanced by bradykinin (Emeis 1992). Two possible explanations for the apparent anomaly with my observations are the relatively long duration of treatment in our study compared to the short term effects of bradykinin infusion in experimental models, and my failure to measure the activity of tissue-type plasminogen activator in addition to mass concentration.

Limitations of this study

Although the results from this study are consistent with previous observations that altered fibrinolysis is associated with an increased risk of myocardial infarction, and that angiotensin converting enzyme inhibitors reduce the risk of subsequent coronary thrombosis in patients with first myocardial infarction, there are a number of limitations to the study. I studied a small, highly selected population for a relatively short period. Only men under the age of 75 years with a first uncomplicated myocardial infarction were included. Patients with previous hypertension or diabetes mellitus, or with any evidence of heart failure, were excluded because these conditions might influence fibrinolysis directly or via associated neurohumoral disturbances. All of the patients were taking a

cardioselective β blocker and aspirin, as is the preferred therapy for patients with myocardial infarction in the absence of any contraindication. Neither β blockers (Hamsten 1985; Wright et al 1994) nor aspirin are thought to influence fibrinolysis. My study did not include any examination of tissue-type plasminogen activator release, such as the venous occlusion test (Wiman et al 1983), which might have provided information on changes in endothelial function. However, a recent study in diabetic and non-diabetic survivors of myocardial infarction found no difference in levels of tissue-type plasminogen activator antigen or plasminogen activator inhibitor type 1 antigen and activity after venous occlusion between patients and controls, despite significant differences prior to venous occlusion (Gray et al 1993b). Lastly, a long-lasting hangover effect from acute infarction which might influence the results of the patient group cannot be excluded, although the blood samples for this study were taken at least 12 weeks after the initial event. Mitigating against an acute phase response persisting are the stable lipid values, the uncomplicated recovery from infarction in this group, and the absence of an order effect in the crossover design. This also argues against a hangover effect from the captopril treatment period for those who received captopril first - consistent with the described endocrine response to captopril withdrawal (Maslowski et al 1981). Previous work has suggested that fibrinolytic parameters are only disturbed in the first few days after infarction and are stable after one week (Gram et al 1987a).

6.6 CONCLUSION

A decrease in the incidence of acute coronary syndromes in those receiving angiotensin converting enzyme inhibitors was a common and unexplained finding in three large placebo-controlled trials in patients with mild left ventricular dysfunction (The SOLVD Investigators 1991; The SOLVD Investigators 1992; Pfeffer et al 1992). This study has shown for the first time that captopril modifies endogenous fibrinolysis in patients with recent uncomplicated myocardial infarction. This may help to explain the reduction in thrombotic risk associated with the use of angiotensin converting enzyme inhibitors.

TABLE 24. FIBRINOLYTIC VARIABLES IN 12 NORMAL MEN AND IN 15 PATIENTS WITH RECENT UNCOMPLICATED MYOCARDIAL INFARCTION AFTER 4 WEEKS PLACEBO AND 4 WEEKS CAPTOPRIL

	Myocardial Infarction group			Estimated median difference, 95% CI, Captopril - Placebo
	Normals	Placebo	Captopril	
t-PA antigen (ng/mL)	9.5** (5.3 to 17.8)	16.0 (9.0 to 34.8)	10.3** (4.0 to 21.8)	-7.3, -4.6 to -10.3
PAI-1 antigen (ng/mL)	8.6 (3.8 to 24.5)	17.3 (3.3 to 36.5)	7.8 (2.5 to 28.3)	-3.1, +1.5 to -8.4
PAI-1 activity (AU/mL)	6.3* (1.9 to 19.0)	13.2 (4.0 to 21.9)	9.0* (2.6 to 29.8)	-2.2, -1.0 to -4.3

t-PA = tissue-type plasminogen activator, PAI-1 = plasminogen activator inhibitor type 1, Values are median (range)

* $p < 0.05$ v MI - Placebo, ** $p = 0.001$ v MI - Placebo

TABLE 25. PEARSON CORRELATION MATRIX FOR FIBRINOLYTIC AND METABOLIC VARIABLES IN ALL SUBJECTS

	t-PA antigen	PAI-1 antigen	PAI-1 activity
PAI-1 antigen	0.484	-	-
PAI-1 activity	0.610*	0.673**	-
Fasting insulin	0.173	0.526	0.591*
Fasting glucose	0.260	0.035	0.392
Total cholesterol	0.259	0.068	0.283
Total triglyceride	0.477	0.424	0.596*
HDL cholesterol	-0.475	-0.460	-0.394

PAI-1 = plasminogen activator inhibitor type 1, t-PA = tissue-type plasminogen activator, HDL = high density lipoprotein

***p < 0.05, **p < 0.01 after Bonferroni correction**

Figure 9. Tissue-type plasminogen activator (t-PA) antigen levels in 15 patients with recent uncomplicated myocardial infarction treated for 4 weeks with placebo and captopril in a double-blind, randomised crossover study

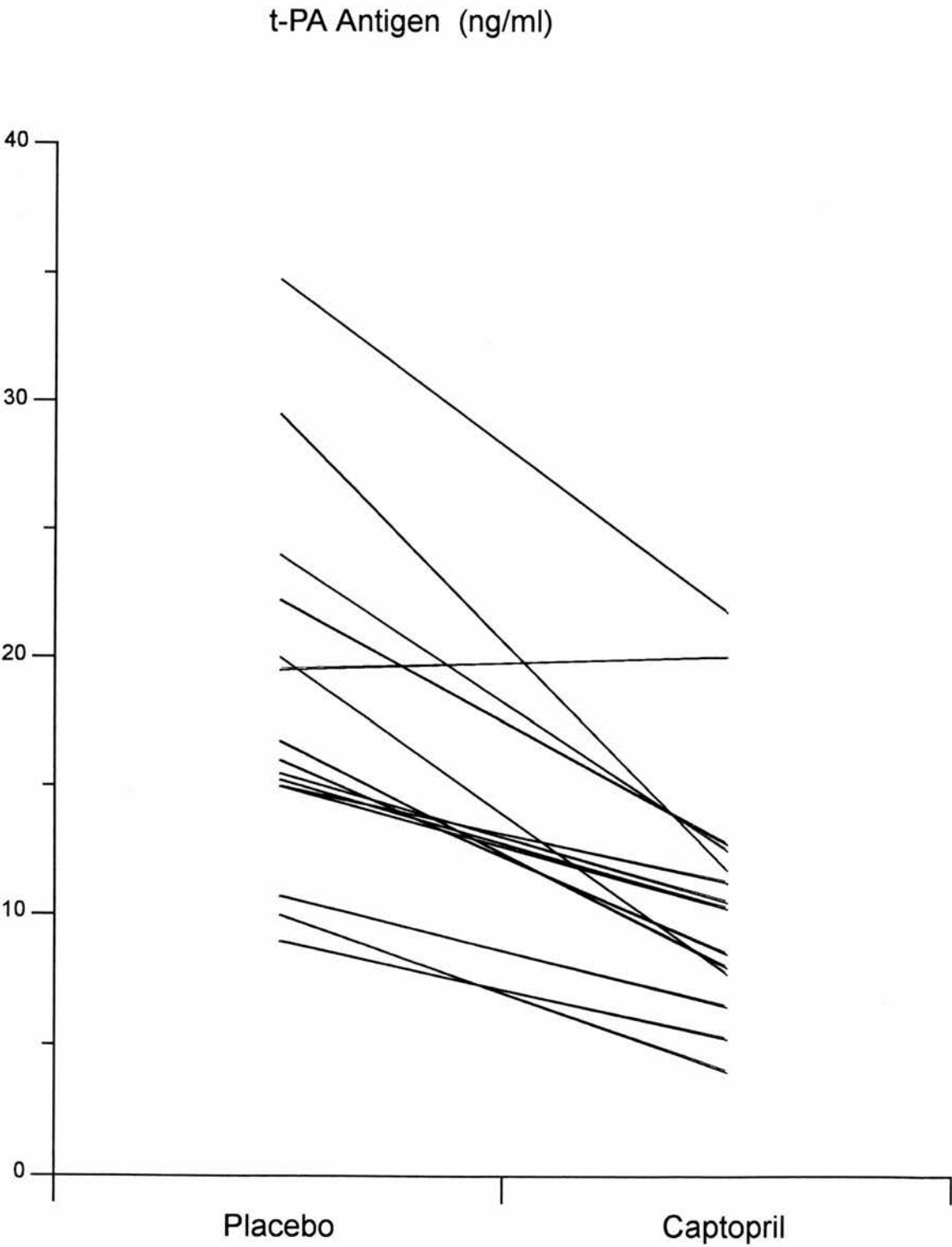


Figure 10. Plasminogen activator inhibitor type 1 (PAI-1) antigen levels in 15 patients with recent uncomplicated myocardial infarction treated for 4 weeks with placebo and captopril in a double-blind, randomised crossover study

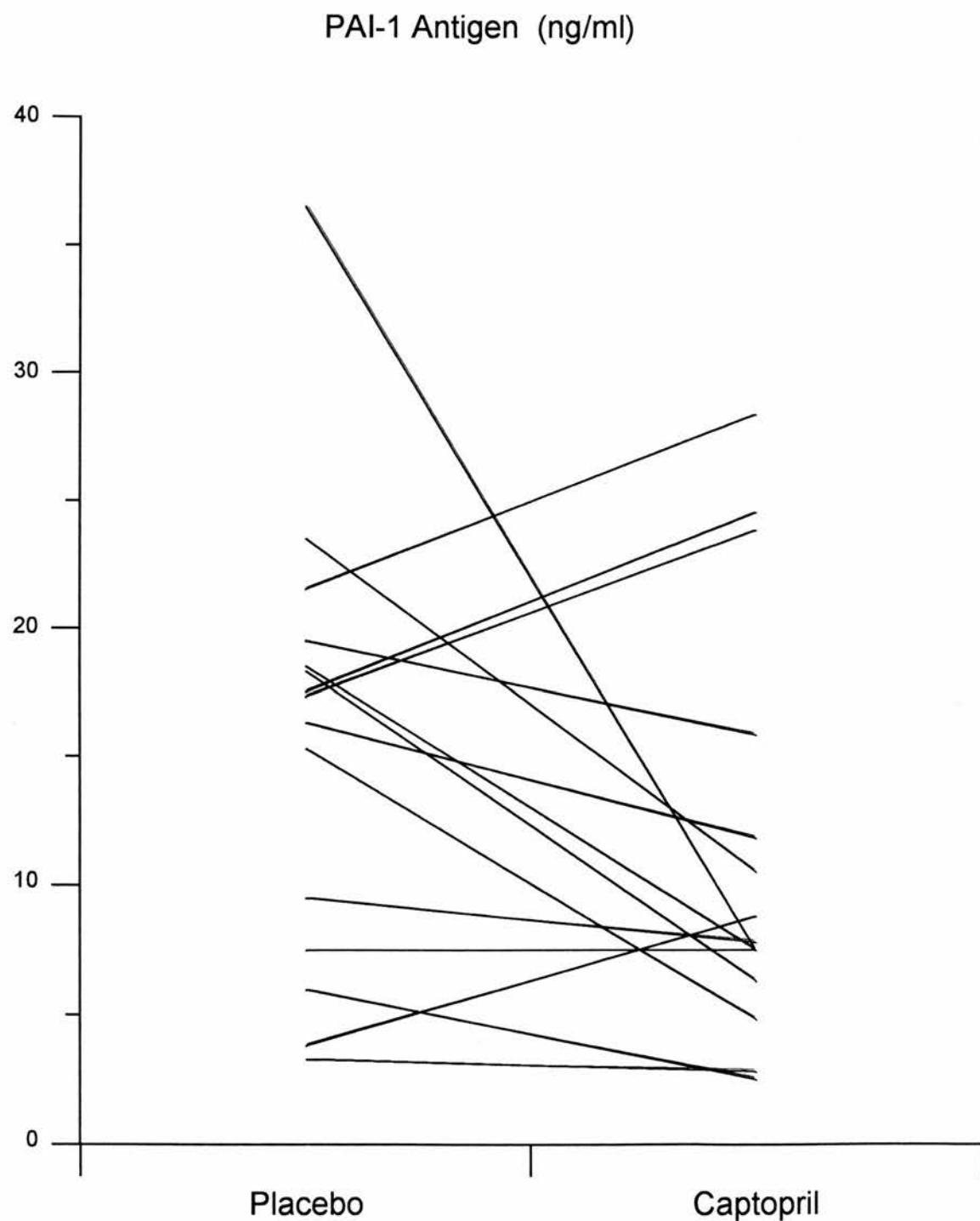
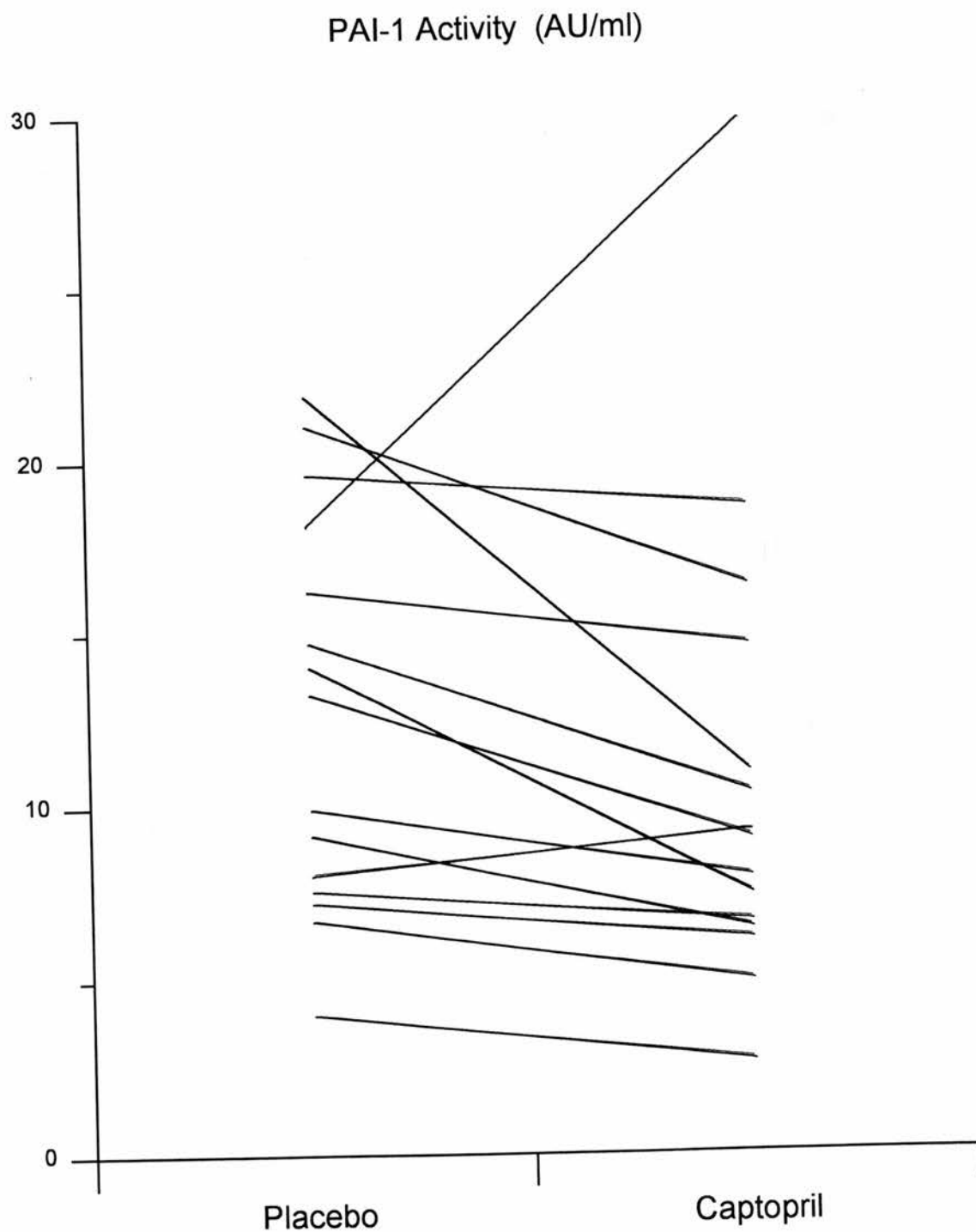


Figure 11. Plasminogen activator inhibitor type 1 (PAI-1) activity levels in 15 patients with recent uncomplicated myocardial infarction treated for 4 weeks with placebo and captopril in a double-blind, randomised crossover study



CHAPTER 7

CONCLUSIONS

Insulin is an important molecule. This is not only suggested by its multiple actions and the problems that develop when it is deficient or when it is present in excess, but also by its remarkable conservation during evolution.

Ischaemic heart disease is an important disease. It usually becomes manifest only after the reproductive period and historically is a feature of modern Western Civilisation. Thus, it can be assumed that ischaemic heart disease has not exerted selection pressure during evolution.

Adaptational and compensatory systems developed during evolution (sympathetic nervous system, renin-angiotensin system, thrombotic and haemostatic systems, and possibly even hyperinsulinaemia and insulin resistance) may exert a detrimental influence upon the well-being of modern man in his middle age and beyond.

This should lead us to view these systems with suspicion when considering the scourge of Western Man - ischaemic heart disease. The work presented in this thesis has explored the hypotheses that disturbances of insulin and of endogenous fibrinolysis are a feature of patients with established ischaemic heart disease and investigated the potential manipulation of these by an angiotensin converting enzyme inhibitor.

Insulin has many diverse actions mediated through different receptors. Insulin may affect the development of ischaemic heart disease either indirectly or directly. Important indirect actions of insulin in this respect include the synthesis of triglyceride rich lipoproteins, increased sympathetic nervous activity, enhanced sodium retention of the kidney, and raised blood pressure. Direct actions of insulin

include stimulating effects on cell growth in those cell types involved in atheroma, inducing atheroma in vivo, and exerting an unfavourable influence on endogenous fibrinolysis. Conversely, it is possible that protection from beneficial actions of insulin may be important in insulin resistant states. This most obviously relates to glucose uptake into tissues, but may also relate to other potentially advantageous actions such as skeletal muscle bed vasodilation. The most likely paradigm is that resistance to some actions of insulin, principally insulin-mediated glucose disposal, lead to a reactive and compensatory hyperinsulinaemia. If the resistance to insulin is not present for other important actions of insulin then this may allow an unregulated expression of those actions and thus have an adverse outcome.

Hyperinsulinaemia has been shown to be a risk factor for developing ischaemic heart disease in three large prospective studies. The role of insulin resistance as a risk factor for developing ischaemic heart disease has not been investigated. Many previous studies have demonstrated that patients with established ischaemic heart disease exhibit hyperinsulinaemia. The importance of the work presented in Chapter 3 was that for the first time I sought to demonstrate whether the nature of the hyperinsulinaemia varied between different states of ischaemic heart disease - chronic stable angina, recent myocardial infarction, and chronic heart failure. I have shown that significant fasting hyperinsulinaemia was a feature only of patients with chronic heart failure. This would explain why fasting hyperinsulinaemia has been an inconsistent finding in previous studies which have not stratified in a similar way. It is probable that the mechanism for this relates to the differing degrees of neurohumoral activation in these groups but I have not

investigated this. I also showed that the degree of stimulated hyperinsulinaemia was less in patients with stable angina and more pronounced, but to a similar degree, in patients with recent myocardial infarction or chronic heart failure. This may again reflect neurohumoral activation, but it suggests to me that stimulated hyperinsulinaemia may be associated with an increased risk of coronary thrombosis. This hypothesis is also consistent with the finding from the primary prospective studies that the most consistent indicator of risk was the stimulated insulin response rather than the fasting level.

Impaired glucose tolerance is relatively common amongst the general population and is associated with both an increased risk of developing ischaemic heart disease and with hyperinsulinaemia and insulin resistance. Amongst patients with ischaemic heart disease there is a much higher prevalence of impaired glucose tolerance, but the degree of associated hyperinsulinaemia had not been studied in this group using recent definitions of glucose tolerance. In Chapter 4, I presented the data obtained from screening a cohort of patients attending the out patient department, none of whom were suspected of having abnormal glucose tolerance. The prevalence of impaired glucose tolerance in this sample was 20% and patients with impaired glucose tolerance had significantly greater hyperinsulinaemia, both fasting and stimulated, than patients with normal glucose tolerance. This suggests that the group with impaired glucose tolerance may have a poorer prognosis. Considering all patients, irrespective of glucose tolerance status, it was possible to show an inverse relationship between fasting insulin concentration and left ventricular ejection fraction. This has not previously been reported and it confirms that fasting

hyperinsulinaemia reflects impaired left ventricular function. This is consistent with the findings in Chapter 3 where it was only the fasting hyperinsulinaemia which discriminated the group of patients with heart failure.

Initial reports from patients with essential hypertension suggested that captopril, an angiotensin converting enzyme inhibitor, improved insulin sensitivity. This may be important because of the need to establish therapies in hypertension which not only lower blood pressure but also reduce the risk of developing ischaemic heart disease. In Chapter 5 the effect of captopril upon hyperinsulinaemia and insulin sensitivity in patients with recent myocardial infarction was described. In a randomised, double-blind comparison with placebo, captopril had no effect upon fasting hyperinsulinaemia, stimulated hyperinsulinaemia, or insulin sensitivity. The use of an angiotensin converting enzyme inhibitor in patients with recent myocardial infarction has been shown to improve prognosis. The data presented suggest that this beneficial effect of angiotensin converting enzyme inhibition is not mediated through insulin sensitivity or hyperinsulinaemia and also indicates that the mechanism underlying hyperinsulinaemia in essential hypertension and ischaemic heart disease may be different.

Over the last decade a number of studies have suggested that components of the endogenous fibrinolytic system may be important in determining risk of thrombotic events. In particular, increased plasma concentrations of tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor type 1 (PAI-1) activity have been associated with an increased risk of myocardial infarction in patients with ischaemic heart disease. The work presented in Chapter 6 sought to

investigate the hypothesis that angiotensin converting enzyme inhibition might convey its benefit of reducing the risk of recurrent acute coronary syndromes in patients with recent myocardial infarction via an effect upon these components of endogenous fibrinolysis. In a double-blind, randomised comparison with placebo, 4 weeks treatment with captopril was associated with a significant decrease in t-PA antigen and in PAI-1 activity. This may help to explain the diminished risk of coronary thrombosis associated with the use of angiotensin converting enzyme inhibitors. However, my initial hypothesis that captopril might exert a favourable effect upon fibrinolysis via altered hyperinsulinaemia or insulin sensitivity was disproved. More recent work has suggested a direct influence of the renin-angiotensin system upon fibrinolysis as a more likely explanation, but my studies did not address this.

Issues which remain to be resolved

Hyperinsulinaemia

The data presented in Chapters 3 and 4 suggest that raised fasting concentrations of immunoreactive insulin are a feature of patients with chronic heart failure and that they are negatively correlated to the left ventricular ejection fraction. It seems most likely that these reflect the neurohumoral activation in heart failure but an intriguing possibility is that in addition they might relate to the abnormalities of peripheral muscle function which have been described in heart failure. Two alternative mechanisms would be impaired peripheral muscle vasodilation in response to insulin or impaired non-oxidative glucose metabolism.

Whilst the prevalence of impaired glucose tolerance amongst patients with ischaemic heart disease has been described before, the data in Chapter 4 raises the possibility that the marked hyperinsulinaemia in this group might point to a worse prognosis.

The lack of an effect of captopril upon hyperinsulinaemia and insulin sensitivity in patients with recent myocardial infarction presented in Chapter 5 raises a number of questions. The most fundamental is whether there are differences in the nature of the hyperinsulinaemia and the insulin resistance present in essential hypertension and in ischaemic heart disease. In addition, the possibility that aspirin might inhibit the arterial vasodilation caused by angiotensin converting enzyme inhibition has been raised and if correct this might also extend to the metabolic effects of angiotensin converting enzyme inhibition.

Fibrinolysis

The most exciting issues which remain to be resolved after completing this work relate to the mechanism by which captopril might influence the endogenous fibrinolytic system. In Chapter 6 I showed that captopril was associated with a significant fall in t-PA antigen and PAI-1 activity. Recent work has shown that short term infusion of angiotensin II causes an increase in PAI-1 but there was a minimal effect upon t-PA. This would help to explain my observation of a fall in PAI-1 with captopril but not the marked reduction in t-PA antigen. Angiotensin converting enzyme inhibition also leads to an increase in bradykinin because of inhibition of the kinase enzyme and bradykinin promotes release of t-PA from the vascular endothelium. However, before formulating a hypothesis to explain the effect of

angiotensin converting enzyme inhibition upon t-PA it is necessary to confront a more fundamental paradox - why raised plasma concentrations of t-PA antigen are associated with an increased incidence of acute coronary thrombosis and stroke.

Measurements in venous plasma of t-PA antigen and t-PA activity are negatively correlated. This is because the majority of t-PA secreted by the vascular endothelium circulates as a complex bound to its rapidly acting inhibitor PAI-1 and is functionally inactive. Thus a high plasma concentration of t-PA antigen indicates that the endothelium has been actively secreting t-PA in response to a stimulus or stimuli. The stimuli may be the presence of intravascular thrombus or of factors which tend to lead to the formation of intravascular thrombus. It seems probable that the reason that increased concentrations of t-PA antigen indicate increased risk is that they reflect the presence of a tendency to the formation of intravascular thrombus. A future thrombotic event might then relate to an overwhelming increase in the pro-thrombotic state or to a failure of endogenous fibrinolysis.

The mechanisms by which angiotensin converting enzyme inhibition was associated with a fall in t-PA antigen are most likely to involve a modification of the stimuli leading to endothelial release of t-PA or a modification of the endothelial response to those stimuli. An example of the former might be that a fall in PAI-1 preceded the fall in t-PA release. Alternatively, the endothelial response might be important. t-PA which is incorporated early into a thrombus is more effective in achieving lysis and therefore an enhanced endothelial response to releasing t-PA might lead to more effective intravascular lysis and overall reduced total endothelial release of t-PA. Increased early endothelial release of t-PA following angiotensin

converting enzyme inhibition might be caused by an increase in local concentrations of bradykinin.

In the latter half of this century considerable effort has been spent in trying to comprehend the mechanisms which lead to the development of ischaemic heart disease. The identification of cholesterol within the atheromatous plaque led to a concentration of resources investigating "the lipid hypothesis." Whilst the role of dyslipidaemia is important, it has become increasingly apparent that other distortions of normal metabolism and other systems including the haemostatic pathways may be very relevant to the development of atheroma and crucial to the precipitation of the acute coronary syndromes. Within the last decade there have been many landmark advances in the management of patients with myocardial infarction: the use of acute thrombolysis and aspirin, the use of beta adrenoceptor antagonists, the use of angiotensin converting enzyme inhibitors, and the use of 3-hydroxy-3 methylglutaryl coenzyme A reductase inhibitors. Despite this, our understanding of this fascinating disease remains incomplete. I hope that the work presented in this thesis has cast some light into the shadows.

CHAPTER 8

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APPENDIX

PUBLISHED WORK ARISING FROM OR RELEVANT TO THIS THESIS

Hyperinsulinaemia in ischaemic heart disease: the importance of myocardial infarction and left ventricular function

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Summary

Elevated circulating insulin levels have been reported in ischaemic heart disease, and may be of aetiological importance. Previous studies have not considered the potential influence of heart failure or of previous myocardial infarction, as opposed to stable angina. We therefore measured the insulin response to a 75 g oral glucose tolerance test in five groups with normal glucose tolerance, comparing normal male controls to men with chronic stable angina, men with recent myocardial infarction (two groups, 3 weeks and 3 months post infarction), and men with chronic severe heart failure. Only patients with chronic heart failure had fasting hyperinsulinaemia, probably reflecting associated neuroendocrine

abnormalities. Stimulated hyperinsulinaemia was present in all patient groups, but was less pronounced and of shorter duration in patients with angina. At 120 min, only patients with heart failure or previous myocardial infarction were hyperinsulinaemic. The degree of stimulated hyperinsulinaemia was not influenced by the presence of heart failure or by the length of time from infarction. Hyperinsulinaemia is associated with impaired peripheral muscle glucose uptake and metabolism, and might contribute to muscular fatigue on exertion in patients with previous myocardial infarction or heart failure.

Introduction

Hyperinsulinaemia has been described in obesity, impaired glucose tolerance (IGT), non-insulin dependent diabetes mellitus, hypertension and ischaemic heart disease (IHD).¹ Insulin may have a direct role in the development of atherosclerosis, or may influence other risk factors for IHD, such as blood pressure and lipoproteins.² The relationship of fasting and stimulated hyperinsulinaemia to IHD has not been clearly defined. Studies in which an oral glucose load was given to patients with IHD have frequently reported stimulated hyperinsulinaemia,³⁻¹⁴ less often fasting hyperinsulinaemia,^{3,12,14} and two studies have failed to show fasting or stimulated hyperinsulinaemia.^{15,16} Earlier studies were limited, as they frequently involved only patients with previ-

ous myocardial infarction and they failed to differentiate the importance of heart failure. Often patients with IGT or diabetes mellitus were not excluded^{4-11,13-15} and patients and controls were not matched for other important confounding variables, including age, body mass index (BMI) or blood pressure.^{3-6,8-16} The administered glucose load has not been the standard 75 g in all studies, and the subsequent blood sampling has sometimes been limited to 60 min.

We measured the immunoreactive insulin (IRI) response to a 75 g oral glucose tolerance test over 120 min in five groups with normal glucose tolerance matched for age, BMI and blood pressure. We compared normal male controls to men with chronic

stable angina, men with myocardial infarction assessed either at 3 weeks or 3 months after the acute event, and men with severe chronic heart failure.

Methods

Patients

Normal controls ($n=22$) were identified at random from the Lothian Health Board register. They had no previous history of hypertension, IHD or family history of diabetes mellitus. They were taking no regular medication, and had a normal 12-lead electrocardiogram. Patients with stable angina ($n=15$) were identified on the basis of a typical history of exertional chest pain and the development of significant electrocardiographic changes (>1 mV ST segment depression 0.06 s after the J point) during treadmill exercise testing. None had any clinical evidence of heart failure. At the time of study they were receiving nifedipine and none were taking beta blockers. The two groups of patients with recent myocardial infarction had an initial diagnosis based upon WHO criteria.¹⁷ One group was studied 3 weeks after the acute event (MI Group I, $n=26$) and one group was studied 12 weeks after the acute event (MI Group II, $n=15$). None of these patients had symptomatic heart failure, and all were taking aspirin. Twenty (77%) of the patients in MI Group I and all those in MI Group II were taking a beta blocker. Patients with chronic heart failure ($n=16$) were free of oedema, but remained severely restricted (all NYHA grade III). At the time of study, the heart failure group were all receiving a loop diuretic with potassium supplements and none were taking an angiotensin converting enzyme inhibitor.

We screened 93 patients attending the out-patient department with established IHD and with fasting plasma glucose <6.7 mmol/l using a 75 g oral glucose tolerance test. Twenty-one (23%) patients were found to have IGT or diabetes mellitus according to WHO criteria (2-h glucose >11 mmol/l = diabetes, >7.8 mmol/l = IGT)¹⁷ and were excluded from this analysis, because both of these conditions have previously been associated with hyperinsulinaemia.¹

Data from 72 patients and 22 normal controls are presented. Details of age, BMI (weight/height²), blood pressure, smoking habit and left ventricular ejection fraction are given in Table 1. The groups were well matched for age, BMI and blood pressure. Ten (63%) of the patients with chronic heart failure were smokers, compared to $<10\%$ of the other groups. Measurement of left ventricular function was made by radionuclide ventriculogram in patients with a

history of myocardial infarction or heart failure. MI Group I and MI Group II had similar and well preserved left ventricular function, whereas the group with NYHA grade III heart failure had significantly decreased left ventricular ejection fraction by comparison with those groups.

Glucose tolerance test

Patients and controls attended the department after a 12-h overnight fast. An indwelling 16G intravenous cannula was inserted into an antecubital vein, and they rested for 30 min. After a baseline blood sample they took 75 g glucose monohydrate in 200 ml water. Further blood samples were taken at 30, 60, 90 and 120 min. Samples were placed on ice. Plasma was separated within 1 h, and stored at -40°C . Each sample was assayed for glucose and IRI. Glucose was measured using a Cobas Bio analyser by the hexokinase method¹⁸ with an inter-assay coefficient of variation of 1.7%. IRI was measured by radioimmunoassay¹⁹ with a coefficient of variation of 6.8–7.5%. This assay does not differentiate between insulin, proinsulin and proinsulin split products.

Statistical analysis

Descriptive data are given as means \pm SEM. Areas under the curve (AUC) were calculated using the trapezium rule. Glucose values are given as arithmetic means (95% CI of mean). Values for insulin and insulin/glucose ratio were compared after logarithmic transformation, and are expressed as geometric means (95% CI of mean). Analysis of variance (ANOVA) was used to assess differences among the means of the groups. Where ANOVA revealed a significant difference between the five groups, further pairwise comparisons were made using Fisher's least-significant-difference test. Statistical analyses used SYSTAT for Windows, Version 5.²⁰ The level of significance was $p<0.05$.

Results

A summary of the results is shown in Table 2

Glucose response to oral glucose

There was no significant difference between the five groups for glucose at either fasting level, 120 min level or in AUC value (Figure 1). Despite all patients having normal glucose tolerance, glucose levels at 120 min tended to be higher in patients with previous MI or heart failure, compared to controls.

Table 1 Baseline characteristics of the five groups

	Controls (n=22)	Stable angina (n=15)	Heart failure (n=16)	MI Group I (n=26)	MI Group II (n=15)
Age (years)	56 ± 1	60 ± 2	60 ± 2	61 ± 2	63 ± 2
BMI (kg/m ²)	24.9 ± 0.6	26.7 ± 0.8	25.3 ± 0.7	25.3 ± 0.6	25.2 ± 0.7
Systolic BP	118 ± 4	127 ± 5	127 ± 5	122 ± 4	127 ± 5
Diastolic BP	79 ± 2	78 ± 3	80 ± 3	76 ± 2	80 ± 3
Smoking	1	1	10	2	1
LVEF	NM	NM	0.20* ± 0.02	0.37 ± 0.02	0.39 ± 0.02

MI, myocardial infarction; BMI, body mass index; LVEF, left ventricular ejection fraction; NM, not measured. Values are mean ± SEM where applicable.

* $p < 0.001$; Student's *t* test vs. both of the MI groups.

Table 2 Plasma glucose and insulin responses to glucose tolerance test

	Controls (n=22)	Stable angina (n=15)	MI Group I (n=26)	MI Group II (n=15)	Heart failure (n=16)	ANOVA <i>p</i> value
Fasting						
Glucose (mmol/l)	5.0 (4.8, 5.2)	5.1 (5.0, 5.3)	5.0 (4.8, 5.2)	5.3 (5.0, 5.5)	5.0 (4.7, 5.4)	0.483
Insulin (mU/l)	5.5 (4.3, 7.0)	7.2 (5.0, 10.2)	6.2 (5.1, 7.5)	5.4 (3.5, 8.1)	11.5 (7.1, 18.8)	0.012
Insulin/ Glucose (mU/mmol)	1.1 (0.8, 1.4)	1.4 (1.0, 2.0)	1.2 (1.0, 1.5)	1.0 (0.7, 1.5)	2.3 (1.4, 3.7)	0.007
120 min						
Glucose (mmol/l)	5.3 (4.8, 5.8)	5.1 (4.6, 5.7)	5.9 (5.4, 6.4)	6.1 (5.4, 6.8)	6.0 (5.4, 6.6)	0.076
Insulin (mU/l)	30.8 (22.0, 43.2)	36.9 (27.8, 49.0)	62.2 (46.8, 82.6)	56.5 (37.2, 85.7)	69.1 (47.9, 99.8)	0.002
Insulin/ Glucose (mU/mmol)	5.9 (4.4, 7.8)	7.3 (5.8, 9.2)	10.8 (8.5, 13.7)	9.5 (6.6, 13.9)	11.9 (8.6, 16.3)	0.004
AUC						
Glucose (min.mmol/l)	791 (728, 855)	894 (823, 966)	848 (804, 892)	846 (787, 906)	837 (758, 915)	0.256
Insulin (min.mU/l)	5041 (3985, 6378)	7377 (6088, 8939)	7951 (6396, 9884)	8050 (5947, 10898)	8998 (6906, 11721)	0.011
Insulin/ Glucose (min.mU/mmol)	6.1 (5.0, 7.5)	8.4 (7.1, 9.8)	9.4 (7.7, 11.6)	9.6 (7.3, 12.6)	10.9 (8.8, 13.6)	0.004

A 75 g oral glucose tolerance test was administered to normal controls and patients with stable angina, patients after acute myocardial infarction studied at 3 weeks or 3 months, and patients with chronic heart failure. AUC, Area under the curve calculated from samples at 0, 30, 60, 90 and 120 min; ANOVA, analysis of variance between the five groups. Values are arithmetic means for glucose and geometric means for insulin and insulin/glucose ratio, with the 95% CI for the means in parentheses.

Insulin response to oral glucose

Plasma insulin levels were significantly different between the five groups for fasting ($p=0.012$), at

120 min ($p=0.002$) and for AUC ($p=0.011$) (Figure 2). Follow-up pairwise comparisons for fasting insulin showed significant differences between patients with heart failure vs. normal controls ($p=0.002$),

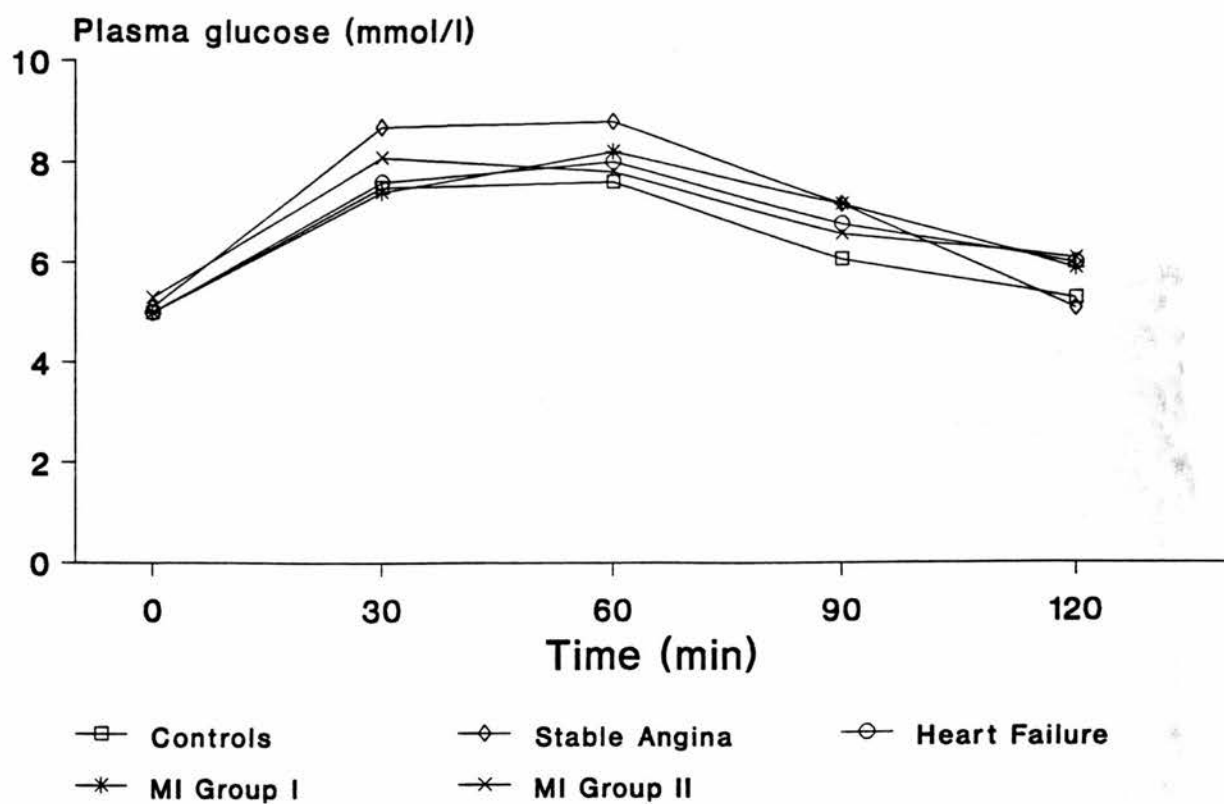


Figure 1. Arithmetic mean plasma glucose concentrations before and after a 75 g oral glucose challenge in the five groups.

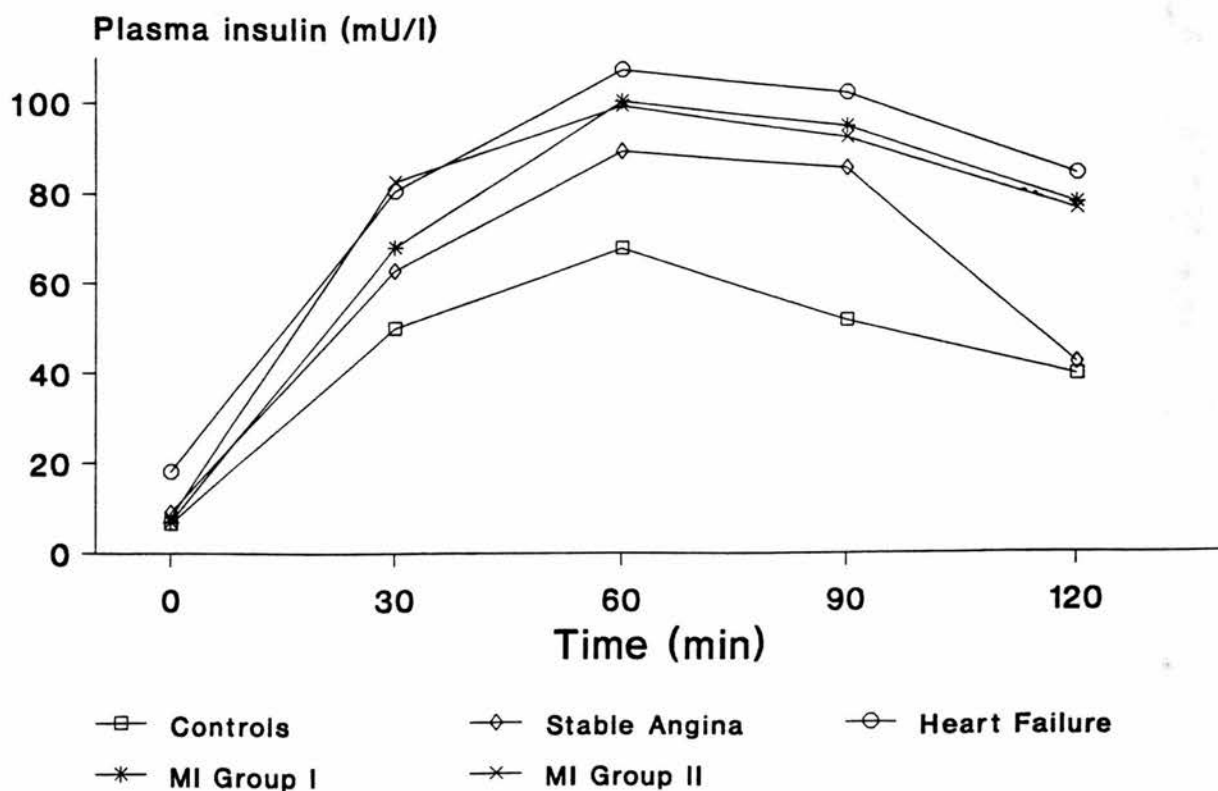


Figure 2. Arithmetic mean plasma insulin concentrations before and after a 75 g oral glucose challenge in the five groups.

MI Group I ($p=0.006$), and MI Group II ($p=0.003$). There were also borderline significant difference between patients with heart failure vs. those with

stable angina ($p=0.06$). Follow-up pairwise comparisons for 120 min levels showed significant differences between normal controls vs. MI Group

($p=0.001$), MI Group II ($p=0.02$), and chronic heart failure ($p=0.001$). In addition, patients with stable angina were significantly different from MI Group I ($p=0.03$) and from chronic heart failure ($p=0.02$). Follow-up pairwise comparisons for AUC showed significant differences between normal controls and all patient groups, controls vs. stable angina ($p=0.03$), MI Group I ($p=0.005$), MI Group II ($p=0.01$), and chronic heart failure ($p=0.001$), but there were no significant differences between patient groups.

Insulin/glucose ratio response to oral glucose

The ratio of plasma insulin/glucose was significantly different between the five groups for fasting ($p=0.007$), at 120 min ($p=0.004$) and for AUC ($p=0.004$) (Figure 3). Pairwise comparisons for fasting ratios showed significant differences between patients with heart failure vs. normal controls ($p=0.001$), stable angina ($p=0.04$), MI Group I ($p=0.005$) and MI Group II ($p=0.001$). Pairwise comparisons showed significant differences for values at 120 min between normal controls vs. MI Group I ($p=0.0012$), MI Group II ($p=0.03$) and chronic heart failure ($p=0.001$), and between stable angina vs. chronic heart failure ($p=0.03$). Pairwise comparisons between the groups for AUC showed significant differences between normal controls vs. stable angina

($p=0.05$), MI Group I ($p=0.003$), MI Group II ($p=0.005$) and chronic heart failure ($p<0.001$).

Discussion

The most important finding of this study is the suggestion that the presence and nature of insulin resistance in patients with IHD may be crucially influenced by previous myocardial infarction or the extent of left ventricular dysfunction. Unlike previous studies,⁴⁻¹⁶ we have taken care to minimize the influence of other conditions known to influence insulin sensitivity¹ by excluding patients with impaired or abnormal glucose tolerance and by matching patient groups and controls for age, BMI and blood pressure. Fasting hyperinsulinaemia was only a feature of patients with chronic heart failure. Stimulated hyperinsulinaemia was present in chronic stable angina, for at least 3 months following recent acute myocardial infarction without clinically significant left ventricular impairment, and in chronic heart failure. Although patients with angina or recent infarction had similar fasting levels of insulin, at 120 min patients with recent infarction had persistently elevated insulin, similar in magnitude to that of the heart failure group and significantly higher than controls, while patients with angina did not differ significantly from controls at this time, but were significantly lower than MI group I or patients with

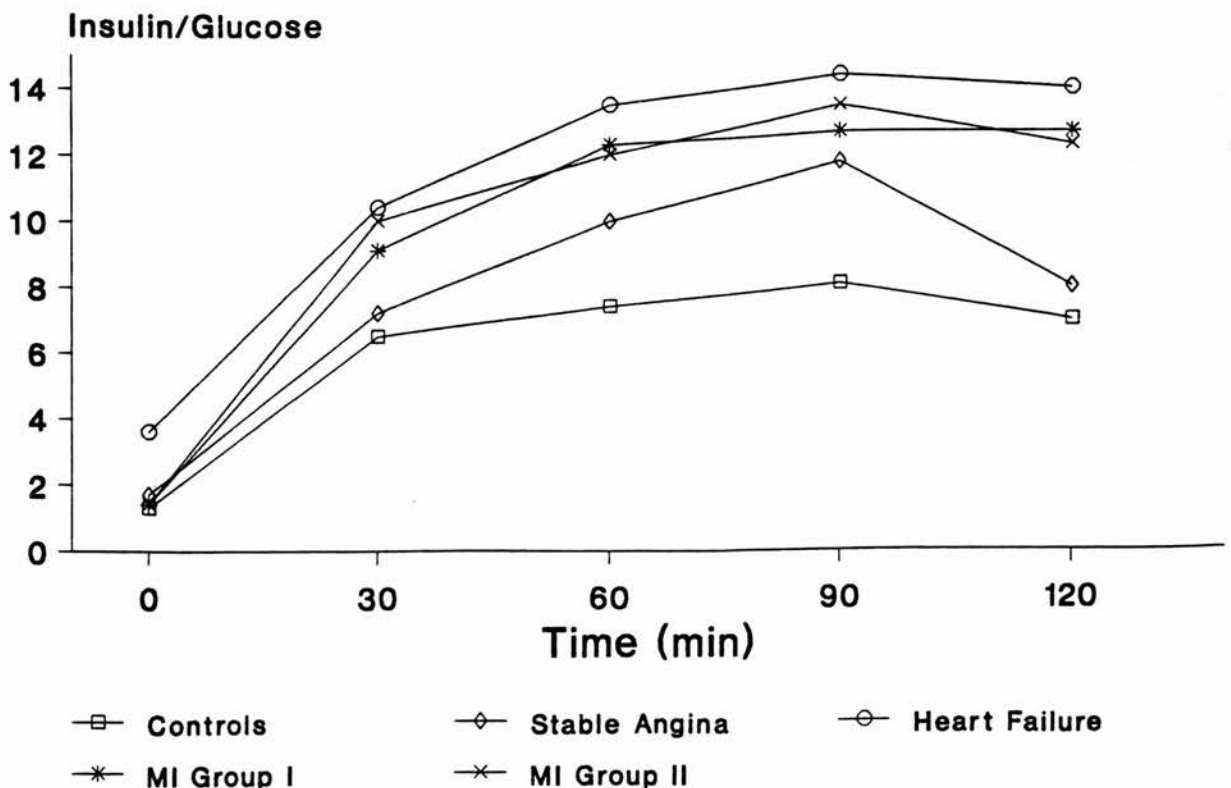


Figure 3. Arithmetic mean plasma glucose/insulin ratios before and after a 75 g oral glucose challenge in the five groups.

chronic heart failure. It may be that the persistent elevation of insulin at 120 min in those with recent myocardial infarction, as opposed to a return to normal insulin levels in those with stable angina, is associated with an increased tendency to occlusive thrombus formation in addition to atheroma. This hypothesis is supported by evidence linking hyperinsulinaemia to impaired endogenous fibrinolysis.²¹ Alternatively, stimulated hyperinsulinaemia may occur as a response to the presence of left ventricular dysfunction, irrespective of the presence of symptoms. If this were the case, then the degree of the stimulated response is not critically dependent upon the extent of ventricular impairment, as the 120 min and AUC values were similar in those with a well-preserved left ventricular ejection fraction and those with severely impaired ejection fraction. The degree of stimulated hyperinsulinaemia in those with recent myocardial infarction did not differ between Group I, studied 3 weeks after the acute event, and Group II, studied 3 months after. We cannot say whether stimulated hyperinsulinaemia was present prior to infarction or developed as a consequence. Three large longitudinal population studies have suggested that hyperinsulinaemia is a risk factor for subsequent myocardial infarction.²²⁻²⁴ Our data would support this hypothesis and in addition, would suggest that fasting hyperinsulinaemia is a late development confined to patients with moderate or severe heart failure.

Low fasting insulin levels and an impaired response to intravenous tolbutamide were found in a previous study in eight patients with heart failure secondary to rheumatic heart disease.²⁵ The effect of an oral glucose load was not tested, and the patients studied were severely ill and bedridden despite treatment with digoxin and diuretics. In end-stage disease it is conceivable that pancreatic hypoperfusion might lead to reduced insulin secretion, but in less severe heart failure there are a number of possible influences on insulin action which might lead to insulin resistance and hyperinsulinaemia. The neuroendocrine response in heart failure leads to increased secretion of catecholamines, cortisol and growth hormone,²⁶ all of which antagonize the peripheral action of insulin. The physical inactivity associated with severe grades of heart failure may also play a role.²⁷ Although there is no evidence from studies in man to support a primary role for hyperinsulinaemia or insulin resistance in the development of heart failure, impaired ventricular function associated with prolonged insulin resistance has recently been described in a rat model of streptozocin-induced diabetes.²⁸ This would be of particular importance because of evidence that angiotensin converting enzyme inhibitors favourably modify insulin resistance.²⁹

Hyperinsulinaemia is an important finding in IHD, because it is associated with dyslipidaemia, hypertension, impaired fibrinolysis and sodium retention by the kidney.^{1,2} In addition, hyperinsulinaemia, particularly in the presence of normal glucose levels, suggests a defect in utilization of substrate by peripheral muscle, as has been shown in diabetes mellitus,³⁰ and this may be relevant to the symptoms of fatigue and diminished effort tolerance which are prevalent in patients with heart failure or after myocardial infarction. Secondly, studies in patients with hypertension have illustrated the potential adverse effects of beta blockers³¹ and beneficial effects of angiotensin converting enzyme inhibitors²⁹ on insulin resistance, and these are classes of drug widely prescribed to patients with IHD.

Study limitations

Nearly all current insulin immunoassays crossreact with proinsulin and 32-33 split proinsulin, and these molecules may form a considerable part of total insulin immunoreactivity after a glucose stimulus in subjects with non-insulin-dependent diabetes mellitus.^{32,33} However, there is no information to suggest that insulin precursor levels are increased in non-diabetic subjects with IHD, and even amongst those with non-insulin-dependent diabetes, it appears that those with mild or diet-controlled disease do not show excess secretion of insulin precursor molecules.³⁴ The stimulated hyperinsulinaemia in some groups may in part reflect relative hyperglycaemia. The elevated ratio of insulin/glucose in those with heart failure or recent myocardial infarction does not support this, however, and suggests that the available insulin was not acting normally to increase glucose uptake, which indicates the presence of insulin resistance.

Although there are no studies of the effect of chronic beta blockade on insulin resistance in patients with IHD, in patients with hypertension, beta blockers have been associated with impaired insulin sensitivity and hyperinsulinaemia.³¹ In our study, the groups taking beta blockers were those with a recent history of myocardial infarction and without heart failure. In MI Group I and MI Group II, fasting levels of insulin were similar to both normal controls and men with stable angina who were not taking a beta blocker. Stimulated levels of insulin in MI Group I and MI Group II were similar to those with heart failure who were not taking a beta blocker. This suggests that beta blockers were not responsible for hyperinsulinaemia in this study.

All of the patients in the group with chronic heart failure were taking loop diuretics with potassium supplements and there are case reports of these being associated with IGT.³⁵ However, the proposed

Table 3 Plasma insulin response to a 75 g oral glucose tolerance test in patients with chronic heart failure—smokers vs. non-smokers

Time (min)	Smokers (n=10)	Non-smokers (n=6)
0	15.6 ± 7.5	19.4 ± 5.8
30	80.3 ± 25.4	80.6 ± 19.7
60	98.2 ± 26.2	112.8 ± 20.3
90	106.7 ± 26.4	99.8 ± 20.4
120	105.7 ± 18.3	71.4 ± 14.2

Plasma insulin in mU/l. Values are mean ± SEM.

mechanism is not through increased insulin release but through inhibition of insulin secretion, and this has been confirmed in an animal study.³⁶ In studies which have examined the effect of frusemide on glucose tolerance and insulin response in patients with hypertension³⁷ and following myocardial infarction,³⁸ there was no effect on fasting or stimulated insulin.

In this study, the group of patients with heart failure was the only group to contain a high proportion of smokers (10/16) and cigarette smoking has been associated in normal volunteers with stimulated hyperinsulinaemia in response to an OGTT and increased insulin resistance.³⁹ However, a comparison of the smokers and non-smokers in the heart failure group (Table 3) showed no significant difference between smokers and non-smokers.

Conclusion

We have shown that, in patients with ischaemic heart disease, fasting hyperinsulinaemia is only present in severe heart failure, whereas stimulated hyperinsulinaemia is prominent amongst those with previous myocardial infarction and those with heart failure, and is also present, but less marked, in those with chronic stable angina.

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Effects of Captopril Therapy on Endogenous Fibrinolysis in Men With Recent, Uncomplicated Myocardial Infarction

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Objectives. This study investigated the effects of captopril therapy on endogenous fibrinolysis in men with recent, uncomplicated myocardial infarction.

Background. Angiotensin-converting enzyme inhibitors reduce the incidence of acute coronary syndromes in patients with mild left ventricular dysfunction after myocardial infarction. Abnormal endogenous fibrinolysis, reflected in increased levels of endogenous tissue-type plasminogen activator (t-PA) antigen and plasminogen activator inhibitor type 1 activity, is associated with an increased risk of myocardial infarction in patients with ischemic heart disease.

Methods. In a randomized, double-blind crossover study beginning 8 weeks after uncomplicated myocardial infarction, patients received 4 weeks of placebo and 4 weeks of captopril (75 mg daily) therapy. At the end of each treatment period, we measured t-PA antigen and plasminogen activator inhibitor type 1 antigen and activity.

Results. Median values in the 15 patients after placebo and in

12 normal men matched for age and body mass index were, respectively, t-PA antigen 16.0 versus 9.5 ng/ml ($p = 0.001$), plasminogen activator inhibitor type 1 antigen 17.3 versus 8.6 ng/ml ($p = 0.29$) and plasminogen activator inhibitor type 1 activity 13.2 versus 6.3 AU/ml ($p = 0.04$). After 4 weeks of treatment with captopril in the 15 patients, the estimated (95% confidence interval) median reduction in t-PA antigen was 7.3 ng/ml (-4.6 to -10.3 ng/ml, $p = 0.001$), in plasminogen activator inhibitor type 1 antigen 3.1 ng/ml ($+1.5$ to -8.4 ng/ml, $p = 0.17$) and in plasminogen activator inhibitor type 1 activity -2.2 AU/ml (-1.0 to -4.3 AU/ml, $p = 0.02$).

Conclusions. Treatment with captopril after uncomplicated myocardial infarction is associated with a significant decrease in elevated levels of t-PA antigen and plasminogen activator inhibitor type 1 activity. This may help to explain the reduction in risk of coronary thrombosis associated with the use of angiotensin-converting enzyme inhibitors.

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Data from three large placebo-controlled studies have indicated that the administration of angiotensin-converting enzyme inhibitors in patients with mild left ventricular dysfunction after myocardial infarction results in a reduced incidence of acute coronary syndromes (1-3). The mechanism by which this is achieved is not clear, but suggestions have included effects on blood pressure, angiotensin II or the arterial intima (4).

Impaired endogenous fibrinolysis is associated with an increased risk of intravascular thrombosis (5). The activity of the fibrinolytic system is reflected in circulating levels of

tissue-type plasminogen activator (t-PA) and its most physiologically important inhibitor, plasminogen activator inhibitor type 1 (6). Increased levels of t-PA antigen have been shown to be a marker of risk for coronary thrombosis in healthy men (7), in patients with angina (8,9) and in patients with myocardial infarction (10) and most recently have been shown to predict long-term mortality in patients with coronary artery disease (11). Elevated levels of plasminogen activator inhibitor type 1 activity have been identified in young survivors of myocardial infarction (12) and were also shown to be associated with an increased risk of reinfarction (13). Studies in normal subjects (14) and in patients with angina (15) have shown a correlation between levels of fasting insulin and both t-PA activator and plasminogen activator inhibitor type 1. This is supported by in vitro evidence that insulin increases the production of plasminogen activator inhibitor type 1 by hepatocytes (16,17). Angiotensin-converting enzyme inhibitors have been shown to improve hyperinsulinemia in hypertensive patients (18) and thus might influence endogenous fibrinolysis through this pathway. Recently it has been shown that an intravenous infusion of angiotensin II results in an increase in plasma levels of plasminogen activator inhibitor type 1 (19),

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Table 1. Details of the Index Myocardial Infarction in the 15 Study Patients

	No. of Pts or Mean Value (range)
Cigarette smoker	3
Family history of IHD	3
Anterior site	8
Inferior site	7
Q wave	14
Streptokinase therapy	14
Peak creatine kinase (IU/liter)	1,900 (476-4,259)
Cardiothoracic ratio	0.48 (0.40-0.55)
LVEF at 6 wk	0.37 (0.28-0.42)

IHD = ischemic heart disease; LVEF = left ventricular ejection fraction; Pts = patients.

providing a link between the renin-angiotensin system and risk of thrombosis and an alternative path by which angiotensin-converting enzyme inhibitors might influence fibrinolysis.

We investigated the hypothesis that the angiotensin-converting enzyme inhibitor captopril might modify endogenous fibrinolysis in men after myocardial infarction that is uncomplicated by clinically manifest heart failure, arrhythmia and recurrent ischemia.

Methods

Subjects. Eighteen men <75 years old and with their first myocardial infarction were recruited 6 weeks after admission to hospital. In addition to a typical history, subjects had to show pathologic Q waves on the electrocardiogram (ECG) or to have had a peak creatine kinase (CK) level >800 IU/liter, or both. Patients with a history of hypertension or diabetes mellitus were excluded. All patients had been taking a cardioselective beta-adrenoceptor blocking agent and 300 mg of aspirin daily from the time of admission to hospital, and no other medication was permitted. None had previously taken an angiotensin-converting enzyme inhibitor. At the time of recruitment a full clinical assessment was made, and a chest radiograph, symptom-limited treadmill exercise test and radionuclide ventriculogram were performed. Any patient with clinical or radiologic signs of heart failure or evidence of ischemia or arrhythmia requiring additional therapy was excluded, and only those considered to have made an uncomplicated recovery from their infarction and without other significant illness were admitted to the study. Three patients were excluded from analysis: one patient whose compliance was <90% by tablet count (75%), one patient whose beta-blocker was withdrawn by his general practitioner during the study and one who moved to a different city during the study and was unwilling to continue with study medication. Details of the index admission to hospital for the 15 patients are given in Table 1.

To provide comparison for fibrinolytic variables, 12 normal men similar in age to the patients were identified at

random from the Lothian Health Board Register. None of these men gave a history of ischemic heart disease, hypertension or diabetes mellitus, and none was taking any medication. They all had a normal physical examination and normal 12-lead ECG.

All subjects gave their oral and written consent to participate in the study, and the study was approved by the Lothian Health Board Ethical Committee.

Study design. The study was designed as a randomized, double-blind, placebo-controlled crossover study. Entry into the study was 8 weeks after the onset of myocardial infarction to avoid any acute-phase response or short-term fluctuation in fibrinolytic function (20). Patients received a test dose of placebo or 6.25 mg of captopril under medical supervision at the beginning of each treatment period, and this was followed by one tablet of placebo or 25 mg of captopril three times daily. After 4 weeks of therapy the patients crossed over to the other treatment arm for a further 4 weeks. No patient had a significant hypotensive response. Measurements of fibrinolytic and other variables were made on one occasion only in normal subjects and at the end of each 4-week treatment period in patients (i.e., at 12 and 16 weeks from the onset of myocardial infarction). Patients fasted from 10 PM the previous night, took their beta-blocker, aspirin and placebo/captopril with a glass of water at 7 AM and attended the outpatient department at 8:30 AM. The patients lay recumbent, and a 16-G intravenous cannula was inserted into a large antecubital vein and flushed with saline solution. Thirty minutes later, 5 ml of blood was drawn and discarded. A further 10 ml of blood was drawn into potassium citrate anticoagulant for fibrinolytic assays; 10 ml into lithium heparin for immunoreactive insulin, urea and electrolytes; 2.5 ml into fluoride oxalate for glucose; and 10 ml into a plain glass tube for serum lipoproteins. Ten minutes later a further 5 ml of blood was drawn and discarded, and an additional 10-ml sample for fibrinolytic assays was drawn into potassium citrate. The samples for fibrinolytic assays and insulin were placed into melting ice. Within 1 h, plasma was separated by centrifugation (20 min at $2,000 \times g$ and -4°C), immediately frozen in dry ice and stored at -40°C .

Assays. All fibrinolytic assays were performed at the same time after completion of the study. Assays for t-PA and plasminogen activator inhibitor type 1 antigen were by a two-site enzyme-linked immunosorbent assay (Biopool AB) and were performed as instructed by the manufacturer, according to the method of Ranby et al. (21) in which plasma samples are incubated in microtiter plates coated with monoclonal antibodies against the relevant antigen, unbound antigens are washed off, and bound antigen is detected by addition of a second specific antibody conjugated to horseradish peroxidase. Standard curves were constructed using purified antigen diluted in plasma to known concentrations. The amount of t-PA and plasminogen activator inhibitor type 1 antigen in samples was deduced by comparing absorbance with the standard curve. The coefficients of variation for

Table 2. Baseline Characteristics of the 12 Normal Men and 15 Male Patients With Recent Myocardial Infarction

	Normal Men	Patients
Age (yr)	57 (2)	63 (2)
Height (m)	1.77 (0.02)	1.78 (0.01)
Weight (kg)	79.5 (2.2)	79.5 (2.2)
BMI (kg/m ²)	25.4 (0.6)	25.2 (0.6)
Pulse (beats/min)	67 (2)	60 (2)
Systolic BP (mm Hg)	120 (4)	124 (5)
Diastolic BP (mm Hg)	80 (3)	77 (3)
Cholesterol (mmol/liter)	6.2 (0.3)	6.9 (0.2)
Triglyceride (mmol/liter)	1.5 (0.2)	2.0 (0.2)
HDL cholesterol (mmol/liter)	1.3 (0.1)	1.1 (0.1)
Glucose (mmol/liter)	4.9 (0.1)	5.2 (0.1)
Insulin (mU/liter)	7.3 (1.8)	7.7 (2.3)

Data presented are mean values (SE). BMI = body mass index; BP = blood pressure; HDL = high density lipoprotein.

repeated measures of t-PA antigen and plasminogen activator inhibitor type 1 antigen in our laboratory were 9.2% and 5.5%, respectively. Plasminogen activator inhibitor type 1 activity was measured by chromogenic assay (Biopool AB) according to the manufacturer's instructions and according to the method first presented by Chmielewska et al. (22). Briefly, a fixed amount of t-PA is added to the plasma sample and allowed to react with the plasminogen activator inhibitor type 1 present. The residual t-PA activity is measured by its ability to catalyze the conversion of plasminogen to plasmin, assessed colorimetrically and compared with standard curves. The plasminogen activator inhibitor type 1 activity is defined as the difference between the amount of t-PA added and the amount of t-PA found. The coefficient of variation for repeated measures of plasminogen activator inhibitor type 1 activity in our laboratory was 5.9%. Total immunoreactive insulin was measured by radioimmunoassay (23). Urea and electrolytes were measured on an autoanalyzer. Total plasma cholesterol and triglyceride levels were determined enzymatically, and HDL cholesterol, using magnesium dextran and plasma glucose, was measured using the hexokinase method (Cobas Bioanalyzer).

Statistical analyses. For fibrinolytic variables, the mean of two samples taken 10 min apart was calculated, and the values are presented as median (range) because of their skewed distribution. Other variables are given as mean values (SE). Comparison of fibrinolytic variables between the normal subjects and patients with myocardial infarction taking placebo was by Mann-Whitney *U* test. Comparison of fibrinolytic variables between the patients with myocardial infarction at the end of each treatment period was by Wilcoxon rank-sum test, and the estimated median and 95% confidence intervals (CI) were calculated using Minitab Release 7 (Minitab Inc.) on a personal computer. Comparison of other variables between groups was by paired or unpaired Student *t* test, as appropriate. To examine relations between hemostatic and metabolic variables for all subjects (normal men and patients with myocardial infarction on placebo), a Pearson correlation matrix using log-transformed data for variables that were not normally distributed (insulin, t-PA, plasminogen activator inhibitor type 1 antigen, plasminogen activator inhibitor type 1 activity) was constructed with Systat for Windows, Version 5 (Systat Inc.) on a personal computer. The significance level for correlations is given after applying the Bonferroni correction for multiple comparisons. All *p* values reported are two-tailed.

Results

Baseline characteristics. There were no significant differences in the baseline characteristics of the 12 normal men and the 15 patients (Table 2).

Fibrinolytic variables. Normal men versus myocardial infarction patients. The t-PA antigen levels at the end of the placebo period were significantly elevated in patients compared with normal men (*p* = 0.001). Plasminogen activator inhibitor type 1 antigen tended to be higher in patients, and plasminogen activator inhibitor type 1 activity was significantly increased (*p* = 0.04) (Table 3).

Patients with myocardial infarction: placebo versus captopril therapy. Nine patients received placebo first, and six received captopril first in the crossover design. There was no evidence of an order effect, and therefore summary results

Table 3. Fibrinolytic Variables in Normal Men and in Patients With Recent Myocardial Infarction After 4 Weeks of Placebo and 4 Weeks of Captopril Therapy

	Normal Value	Myocardial Infarction Group		Estimated Median Difference (95% CI, captopril - placebo)
		Placebo	Captopril	
t-PA antigen (ng/ml)	9.5* (5.3 to 17.8)	16.0 (9.0 to 34.8)	10.3* (4.0 to 21.8)	-7.3 (-4.6 to -10.3)
PAI-1 antigen (ng/ml)	8.6 (3.8 to 24.5)	17.3 (3.3 to 36.5)	7.8 (2.5 to 28.3)	-3.1 (+1.5 to -8.4)
PAI-1 activity (AU/ml)	6.3† (1.9 to 19.0)	13.2 (4.0 to 21.9)	9.0† (2.6 to 29.8)	-2.2 (-1.0 to -4.3)

**p* = 0.001 and †*p* < 0.05 versus myocardial infarction (placebo therapy). Data presented are median values (range). PAI-1 = plasminogen activator inhibitor type 1; t-PA = tissue-type plasminogen activator.

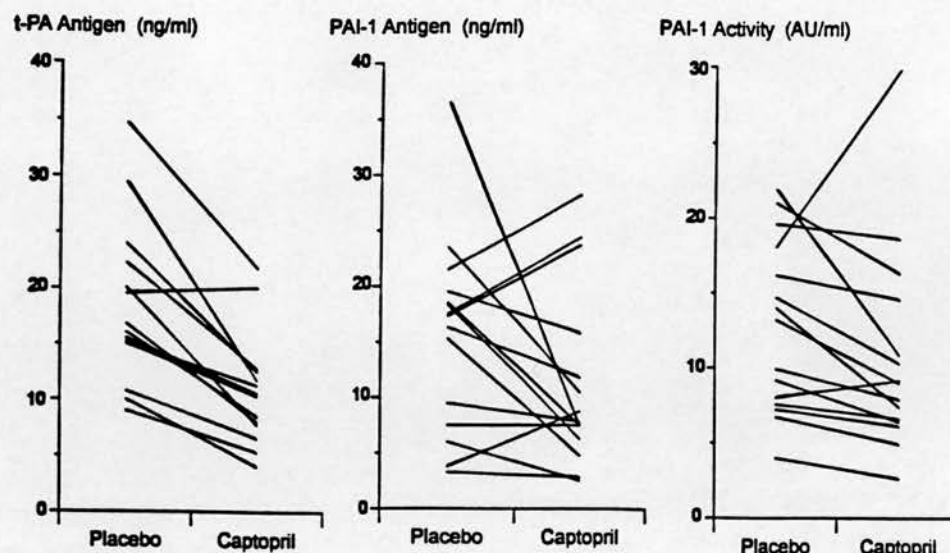


Figure 1. Tissue-type plasminogen activator (t-PA) antigen levels and plasminogen activator inhibitor type 1 (PAI-1) antigen and activity levels in 15 patients with recent, uncomplicated myocardial infarction treated for 4 weeks with placebo and captopril in a double-blind, randomized crossover study.

are presented combined in Table 3; individual results are given in Figure 1.

Tissue-type plasminogen activator antigen. After 4 weeks of therapy with captopril compared with 4 weeks of placebo, there was a highly significant decrease in t-PA ($p = 0.001$), with a median reduction of 46% (95% CI -29% to -64%). Fourteen patients showed a reduction, and one showed a small increase.

Plasminogen activator inhibitor type 1 antigen. After captopril therapy compared with placebo therapy, 10 patients showed a decrease in plasminogen activator inhibitor type 1 antigen, one patient showed no change, and four patients showed an increase. There was no overall significant effect, although there was a median reduction of 18% (+9% to -49%, $p = 0.17$).

Plasminogen activator inhibitor type 1 activity. After captopril therapy, there was a significant reduction in plasminogen activator inhibitor type 1 activity ($p = 0.02$), with a median reduction of 17% (-8% to -33%); 13 patients showed a reduction in plasminogen activator inhibitor type 1 activity, and two showed an increase.

Other variables. Patients with myocardial infarction: placebo versus captopril therapy. After 4 weeks of therapy with placebo compared to 4 weeks of captopril, there was no significant effect on blood pressure or plasma urea, electrolyte, lipoprotein, glucose or insulin levels (Table 4).

Relation between metabolic and hemostatic variables for all subjects. Based on combined results from normal men and patients with myocardial infarction treated with beta-blockers, aspirin and placebo, Table 5 shows the Pearson correlation coefficients between fibrinolytic variables and plasma glucose, insulin and lipid levels. After adjustment for multiple comparisons, the correlations between t-PA antigen and plasminogen activator inhibitor type 1 activity ($r = 0.61$, $p = 0.027$) and between plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor type 1 activity ($r = 0.673$, $p = 0.005$) remained significant. Plasminogen activator inhibitor type 1 activity also showed significant

correlations with fasting insulin ($r = 0.591$, $p = 0.041$) and with fasting triglyceride ($r = 0.596$, $p = 0.037$) levels.

Discussion

Episodes of unstable angina or further infarction have a considerable influence on prognosis after a first myocardial infarction. The balance between thrombosis and fibrinolysis is central to the evolution of these acute coronary syndromes. Our results have shown that there is abnormal activation of the endogenous fibrinolytic system, as shown by elevated levels of t-PA antigen and plasminogen activator inhibitor type 1 activity in male survivors of uncomplicated acute myocardial infarction. In addition, we have shown in a double-blind, placebo-controlled, randomized crossover study that 4 weeks of treatment with captopril (75 mg daily) is associated with a significant decrease in t-PA antigen and plasminogen activator inhibitor type 1 activity. Angiotensin-converting enzyme inhibitors have not previously been reported to have an effect on fibrinolytic variables, and this may help to explain why their use is associated with a reduction in risk of coronary thrombosis.

Table 4. Effect of 4 Weeks of Placebo and 4 Weeks of Captopril Therapy on Other Variables

	Placebo	Captopril
Systolic BP (mm Hg)	117 (4)	114 (4)
Diastolic BP (mm Hg)	69 (3)	68 (4)
Urea (mmol/liter)	6.3 (0.3)	6.5 (0.4)
Sodium (mmol/liter)	141 (1)	141 (1)
Potassium (mmol/liter)	4.1 (0.1)	4.1 (0.1)
Creatinine (μ mol/liter)	105 (3)	105 (3)
Cholesterol (mmol/liter)	6.9 (0.2)	6.8 (0.2)
Triglyceride (mmol/liter)	2.0 (0.2)	2.2 (0.3)
HDL cholesterol (mmol/liter)	1.1 (0.1)	1.1 (0.1)
Glucose (mmol/liter)	5.3 (0.1)	5.2 (0.2)
Insulin (mU/liter)	7.7 (2.3)	6.8 (1.4)

Data presented are mean values (SE). Abbreviations as in Table 2.

Table 5. Pearson Correlation Matrix for Fibrinolytic and Metabolic Variables in All Subjects

	t-PA Antigen	PAI-1 Antigen	PAI-1 Activity
PAI-1 antigen	0.484	—	—
PAI-1 activity	0.610*	0.673†	—
Fasting insulin	0.173	0.526	0.591*
Fasting glucose	0.260	0.035	0.392
Total cholesterol	0.259	0.068	0.283
Total triglyceride	0.477	0.424	0.596*
HDL cholesterol	-0.475	-0.460	-0.394

* $p < 0.05$, † $p < 0.01$ after Bonferroni correction. Abbreviations as in Tables 2 and 3.

Endogenous fibrinolysis in ischemic heart disease. Elevated levels of t-PA antigen at baseline were associated with a greater risk of subsequent myocardial infarction in the Physicians' Health Study (7). Two studies have shown that the risk of subsequent cardiovascular events in patients with unstable angina was predicted by t-PA antigen but not by plasminogen activator inhibitor type 1 activity (8,9), and in a study of patients with previous myocardial infarction, those with reinfarction had higher t-PA antigen but lower t-PA activity (10). A recent study in 213 patients with coronary artery disease has shown that concentrations of t-PA antigen predict mortality over a 7-year follow-up (11). An earlier study of young survivors of myocardial infarction (12) also found higher levels of t-PA antigen and plasminogen activator inhibitor type 1 activity than control subjects, and a greater risk of further infarction was predicted by increased levels of plasminogen activator inhibitor type 1 activity in a study in which tissue-type plasminogen activator antigen was not reported (13). Thus, it has been a consistent finding that t-PA antigen and plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor type 1 activity are all elevated in patients with ischemic heart disease, whereas t-PA activity is decreased. It should be noted that the results of assays that measure levels of antigen include inactive complexes of t-PA with plasminogen activator inhibitor type 1, and therefore it is valuable to have measures of both antigen and activity.

It might initially be thought that elevated levels of t-PA antigen would suggest a decreased risk of thrombosis by indicating a more active fibrinolytic system. One hypothesis that could help to explain these observations is that increased secretion of these molecules, measured by the levels of antigen detected, reflects a response to stimuli that promote thrombosis and that it is these that increase the risk of subsequent thrombotic occlusive events. This hypothesis is supported by evidence that both thrombosis and pharmacologic thrombolysis increase circulating levels of plasminogen activator inhibitor type 1 (24) and the expression of the plasminogen activator inhibitor type 1 gene within the endothelium (25). It is likely that increased circulating plasminogen activator inhibitor type 1 stimulates release of t-PA, which is supported by the strong correlation between t-PA

antigen and plasminogen activator inhibitor type 1 activity seen in this study and reported by others (26). Short-term variations in endogenous fibrinolytic activity may be best assessed by plasminogen activator inhibitor type 1 activity, because this is the predominant physiologic governor of t-PA action. Plasminogen activator inhibitor type 1 exhibits marked diurnal fluctuation (27), whereas t-PA has much less diurnal change, which may have implications for clinical studies and help to explain why levels of t-PA antigen predict risk of future clinical events (7-10), and why results for plasminogen activator inhibitor type 1 are less consistent (8,9,13).

Possible mechanisms in this study. In our study, the influence of captopril therapy on fibrinolytic function occurred despite there being no effect on plasma insulin, glucose, electrolyte and lipoprotein levels. It is important to note, however, that, as previously reported (14,15), there was a positive correlation between plasminogen activator inhibitor type 1 activity and both fasting insulin and triglyceride levels, although each accounts for <40% of the variability of plasminogen activator inhibitor type 1 activity. It may be that the lack of a more marked effect on plasminogen activator inhibitor type 1 in this study reflects the absence of a change in metabolic variables with captopril therapy, but at the same time our results indicate that factors other than these must be important in the regulation of both plasminogen activator inhibitor type 1 and t-PA.

The small decrease in blood pressure with captopril therapy, which was not significant in this study, was comparable to that reported in the Survival and Ventricular Enlargement (SAVE) and Studies of Left Ventricular Dysfunction (SOLVD) studies (1-3). Although angiotensin-converting enzyme inhibitors have been shown to reduce insulin levels in hypertensive subjects (18), this effect has not been found in all groups (28). Recently it has been suggested that the renin-angiotensin system may play a more direct role in the control of endogenous fibrinolysis (19). In a study in hypertensive subjects, an intravenous infusion of angiotensin II caused a significant increase in plasminogen activator inhibitor type 1 antigen with no effect on t-PA antigen (19). Because angiotensin-converting enzyme inhibitors inhibit the formation of angiotensin II, they might be expected to cause a decrease in levels of plasminogen activator inhibitor type 1, but this would not explain the decrease in t-PA. Angiotensin-converting enzyme inhibitors also influence the kinin system and lead to increased levels of bradykinin (29). Tissue-type plasminogen activator is primarily secreted from the vascular endothelium, from which its release is enhanced by bradykinin (30). Two possible explanations for the apparent anomaly with our observations are the duration of treatment in our study compared with the short-term effects of bradykinin infusion in experimental models and our failure to measure the activity of t-PA in addition to mass concentration.

Study limitations. Although our results are consistent with previous observations that altered fibrinolysis is asso-

ciated with an increased risk of myocardial infarction and that angiotensin-converting enzyme inhibitors reduce the risk of subsequent coronary thrombosis in patients with first myocardial infarction, there are a number of limitations to our study. We studied a small, highly selected population for a relatively short period. We included only men <75 years old with a first uncomplicated myocardial infarction. Patients with previous hypertension or diabetes mellitus or with any evidence of heart failure were excluded because these conditions might influence fibrinolysis directly or through associated neurohumoral disturbances. All of our patients were taking a cardioselective beta-blocker and aspirin, as is the preferred therapy for patients with myocardial infarction in the absence of any contraindication. Neither beta-blockers (12,31) nor aspirin is thought to influence fibrinolysis.

Our study did not include any examination of t-PA release, such as the venous occlusion test (32), which might have provided information on changes in endothelial function. However, a recent study in diabetic and nondiabetic survivors of myocardial infarction found no difference in levels of t-PA antigen or plasminogen activator inhibitor type 1 antigen and activity after venous occlusion between patients and control subjects despite significant differences before venous occlusion (33). Finally, we cannot exclude a long-lasting hangover effect from acute infarction that might influence the results of the patient group, although the blood samples for this study were taken at least 12 weeks after the initial event. Mitigating against an acute-phase response persisting are the stable lipid values, the uncomplicated recovery from infarction in our group and the absence of an order effect in the crossover design. This also argues against a hangover effect from the captopril treatment period for those who received captopril first, consistent with the described endocrine response to captopril withdrawal (34). Previous work has suggested that fibrinolytic variables are only disturbed in the first few days after infarction and are stable after 1 week (20).

Conclusions. A decrease in the incidence of acute coronary syndromes in those receiving angiotensin-converting enzyme inhibitors was a common and unexplained finding in three large placebo-controlled trials in patients with mild left ventricular dysfunction (1-3). This study has shown for the first time that captopril modifies endogenous fibrinolysis in patients with recent uncomplicated myocardial infarction. This may help to explain the reduction in thrombotic risk associated with the use of angiotensin-converting enzyme inhibitors.

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*Short communication***The long-term effects of metoprolol and epanolol on tissue-type plasminogen activator and plasminogen activator inhibitor 1 in patients with ischaemic heart disease****R. A. Wright¹, A. M. Perrie², F. Stenhouse¹, K. G. M. M. Alberti³, R. A. Riemersma¹, I. R. MacGregor², N. A. Boon¹**¹ Cardiovascular Research Unit, University of Edinburgh, Edinburgh, UK² Scottish National Blood Transfusion Service, Headquarters Laboratory, Edinburgh, UK³ Department of Medicine, University of Newcastle Upon Tyne, Newcastle upon Tyne, UK

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Abstract. This double-blind, randomized parallel group study investigated the effect of 6 months β -adrenoceptor antagonist therapy with either metoprolol (β_1 -selective without intrinsic sympathomimetic activity [ISA]) or epanolol (β_1 -selective with ISA) on markers of endogenous fibrinolysis in 20 patients with chronic stable angina receiving concurrent treatment with nifedipine.

Neither drug had an effect on tissue-type plasminogen activator or plasminogen activator inhibitor type 1 (PAI-1). A significant correlation between fasting insulin and PAI-1 has previously been described and was confirmed in this study. The group treated with metoprolol showed a significant rise in fasting insulin after 6 months with no change in PAI-1.

This suggests that the previously described link between these two may not be causal.

Key words: Metoprolol, Epanolol, Ischaemic heart disease; tissue-type plasminogen activator, plasminogen activator inhibitor type 1

Tissue-type plasminogen activator (t-PA) [1, 2] and plasminogen activator inhibitor type 1 (PAI-1) [3, 4] have been implicated in the incidence and recurrence of myocardial infarction. Beta blockers are effective in the secondary prevention of myocardial infarction [5] and are also widely prescribed to patients with angina and hypertension. The long term effects of β -adrenoceptor blockade upon t-PA and PAI-1 are unknown, although a possible benefit has been suggested [6], with the caveat that β -adrenoceptor selectivity or the presence of intrinsic sympathomimetic activity (ISA) might be necessary.

This study investigated the effect of 6 months treatment with either metoprolol, a β_1 -selective adrenoceptor antagonist, or epanolol, a β_1 -selective adrenoceptor antagonist with ISA of approximately 20% that of isoprenaline [7], on

t-PA and PAI-1 antigen levels in 20 patients with stable angina. Fasting plasma lipids, glucose and insulin were also measured at the beginning and end of the treatment period.

Subjects and methods

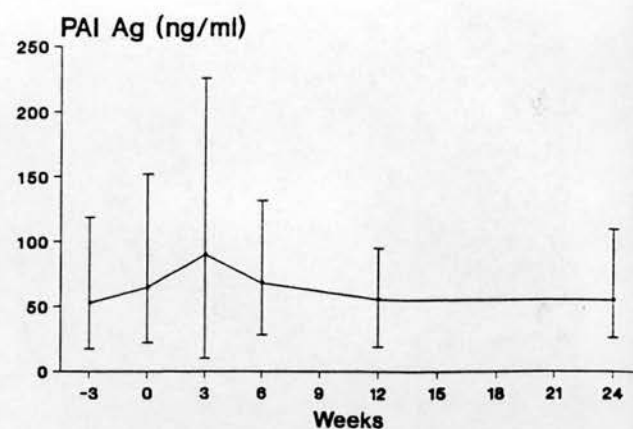
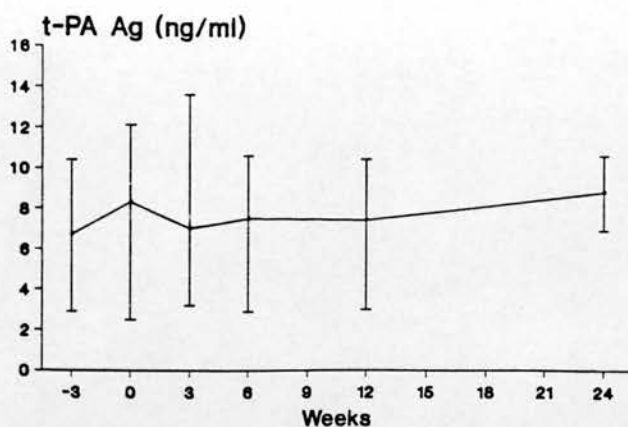
Twenty male patients who had chronic stable angina with a typical history and a positive treadmill exercise test (>0.1 mV ST segment depression on a 12 lead electrocardiogram) were recruited. Patients with a history of hypertension or diabetes mellitus were excluded.

Subjects attended between 08.30 and 10.00 h having fasted for at least 10 h and lay recumbent for 30 min prior to a blood sample being drawn from an antecubital vein with a 19G needle and without venous stasis. Samples were immediately placed into melting ice and within 1 h they were centrifuged at 4°C, 2000 G for 20 min. Plasma was collected, immediately frozen and stored at -40°C . Three weeks later this procedure was repeated to provide a second baseline measure. At this time (week 3), patients were randomised to receive either metoprolol 100 mg twice daily and placebo once daily, or epanolol 200 mg once daily and placebo twice daily. Further visits were made at 6, 9, 15 and 27 weeks. Patients received nifedipine and sublingual GTN throughout the study. The mean (SD) age (60(6) v 54(10) years, NS) and duration of angina (4(5) v 2(4) years, NS) were similar in the metoprolol and epanolol groups respectively. Two patients in each group had a history of previous myocardial infarction.

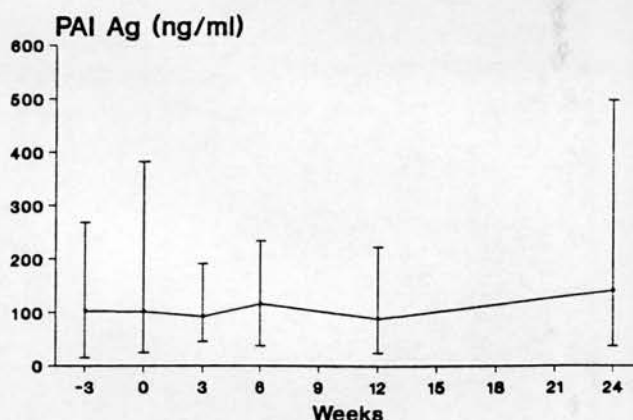
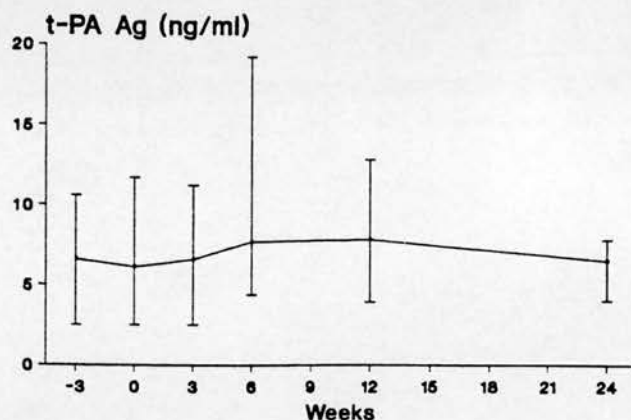
PAI-1 antigen and t-PA antigen were determined using enzyme-linked immunosorbent assays [8, 9]. Total cholesterol and triglycerides were determined enzymatically, HDL cholesterol using Mg^{2+} dextran, plasma glucose by the hexokinase method and total immunoreactive insulin by radioimmunoassay [10].

Statistical methods

Data are expressed as mean (range) for fibrinolytic variables and mean (SEM) for all others. Data which were not normally distributed were log transformed prior to statistical testing. The baseline values of t-PA and PAI-1 for the 2 groups were compared using Student's *t*-test for unpaired data. To assess the effect of the 2 beta blockers, analyses were made separately for the 2 groups. Repeated measures analysis of variance was used to compare PAI-1 and t-PA at the end of the baseline (week 3), and at 6, 9, 15 and 27 weeks treatment. Student's *t*-test for paired data was used to compare other variables measured at 3 weeks and 27 weeks. To assess the influences of BMI and fasting glucose, insulin and lipoproteins upon fibri-



1 Mean (range)



2 Mean (range)

Fig. 1. The effect of 6 months treatment with metoprolol on tissue-type plasminogen activator antigen (t-PA Ag) and plasminogen activator inhibitor-1 antigen (PAI-1 Ag) after 2 baseline measures at week -3 and week 0

Fig. 2. The effect of 6 months treatment with epanolol on tissue-type plasminogen activator antigen (t-PA Ag) and plasminogen activator inhibitor-1 antigen (PAI-1 Ag) after 2 baseline measures at week -3 and week 0

nolytic function at baseline, the results for all patients were combined and Pearson correlation coefficients were calculated. The 2 way level of significance was taken as $P < 0.05$.

Results

For the metoprolol and the epanolol groups respectively baseline levels of t-PA antigen (8.3 (2.5–12.1) vs 6.1 (2.5–11.7) ng·ml⁻¹, NS) and PAI-1 antigen (65 (18–152) vs 101 (24–383) ng·ml⁻¹, NS) were similar. Neither metoprolol (Fig. 1) nor epanolol (Fig. 2) had a significant effect on t-PA antigen or PAI-1 antigen over the 6 month period.

The mean values of the other measured parameters are given in Table 1. After 6 months both beta blockers had induced a significant fall in resting heart rate which was less marked with epanolol, in keeping with its partial agonist activity. There was no significant effect of either drug on blood pressure, body mass index, total cholesterol,

rol, HDL cholesterol or serum triglycerides. There was a tendency for fasting glucose to rise in both groups (NS). The metoprolol group showed a significant rise in fasting immunoreactive insulin ($P < 0.05$) but there was no change with epanolol.

At baseline, there were significant positive correlations for PAI-1 with fasting insulin ($r = 0.59$, $P < 0.01$) and BMI ($r = 0.49$, $P < 0.05$) and for t-PA with fasting cholesterol ($r = 0.53$, $P < 0.05$) and triglyceride ($r = 0.49$, $P < 0.05$).

Discussion

In this study there was no effect of long-term treatment with beta blockers on markers of endogenous fibrinolysis in patients with ischaemic heart disease. There was no difference between metoprolol (β_1 -selective, without ISA) and epanolol (β_1 -selective, with ISA). The baseline measurements confirmed previously described correlations between t-PA and fasting cholesterol and triglycerides [11], and between PAI-1 and fasting insulin and body mass index [11]. Neither drug affected body mass index, fasting lipoproteins or glucose, but metoprolol was associated with a significant rise in fasting insulin, a finding previously described in hypertensives [12]. Despite the increase in fasting insulin, there was no effect upon PAI-1. Thus, the link between fasting hyperinsulinaemia and impaired fibrinolysis may not be a direct one. There was no change in fasting lipids in this study, possibly

Table 1. The effect of 6 months treatment with metoprolol or epanolol on cardiovascular risk factors in 2 groups of patients with chronic stable angina

	Metoprolol group (n = 10)		Epanolol group (n = 10)	
	Baseline	6 months	Baseline	6 months
Body mass index (kg/m ²)	27.5 (3.1)	27.9 (2.9)	27.4 (4.2)	27.5 (4.3)
Pulse (bpm)	79 (15)	59 (5)***	76 (7)	67 (5)**
Systolic blood pressure (mmHg)	124 (18)	122 (16)	132 (19)	123 (10)
Diastolic blood pressure	76 (11)	75 (6)	84 (13)	82 (8)
Total cholesterol (mmol/l)	7.1 (1.2)	7.1 (0.7)	6.7 (1.0)	7.1 (0.6)
HDL cholesterol (mmol/l)	1.2 (0.3)	1.1 (0.2)	1.1 (0.2)	1.2 (0.2)
Triglycerides (mmol/l)	2.2 (0.9)	2.1 (1.0)	2.0 (1.0)	2.8 (1.8)
Fasting glucose (mmol/l)	5.0 (0.4)	5.5 (0.8)	5.1 (0.3)	5.3 (0.4)
Fasting insulin (mu/l)	7.2 (3.6)	15.0 (15.7)*	16.6 (8.6)	14.4 (7.8)

Values are mean (SD) * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

because both drugs investigated were β_1 -selective [13]. As both total fasting cholesterol and triglyceride were positively correlated with t-PA at baseline, it is possible that β -adrenoceptor blockade might influence fibrinolytic variables in a situation in which lipoproteins were also altered.

In the one previous study [14] examining the effect of β -adrenoceptor blockade on fibrinolysis in patients with ischaemic heart disease, which predated assays of t-PA and PAI-1, alprenolol (a nonselective beta blocker with ISA) prolonged euglobulin clot lysis time at rest but did not influence the response to exercise. Metoprolol caused no change in PAI-1 in 10 normal subjects [15], but in 15 hypertensive patients it caused a decrease [16]. In 15 patients with hypertension, the long-acting, non-selective beta blocker nadolol had no effect upon t-PA, PAI-1 or platelet aggregability [17]. The duration of treatment in these studies has not exceeded 3 weeks, whilst beta blockers are prescribed for long periods, and often in the hope of preventing long-term thrombotic complications.

Limitations of this study

Although we included more subjects than previous patient studies, the wide range of values for the fibrinolytic parameters in this study limited the power to show a significant effect. However, the figures illustrate well the complete absence of any effect with either agent. After the randomisation code was broken, it was apparent that the baseline fasting insulin concentrations were lower in the metoprolol group. This may explain the tendency for the baseline PAI-1 levels to be lower in this group. The difference at baseline might suggest that the epanolol group were initially more insulin resistant, but there is no reason to believe that this would alter the response of fibrinolysis to beta blockade.

Our subjects maintained treatment with nifedipine throughout, so our results were obtained in the presence of a calcium antagonist. However, nifedipine does not alter t-PA antigen and activity in normal volunteers [18]. Only 20% of our patient group had suffered a previous myocardial infarction, and there may be differences in fibrinolytic function between patients with angina who suffer myocardial infarction and those who do not [11].

Conclusion

Long-term administration of cardioselective beta blockade to patients with chronic stable angina is not associated with any alteration in t-PA or PAI-1. The presence of ISA did not confer any benefit. After 6 months treatment with metoprolol there was a rise in fasting insulin with no change in levels of plasminogen activator type 1, suggesting that the previously described link between basal insulin secretion and impaired fibrinolysis is not causal.

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